PEARL

The Journal of HEREDITY

GUY N. COLLINS

Linkage of Waxy-purple in Maize Confirmed Gene Theory

Are Viruses "Free Genes"?

Embryo-Rejuvenation in Citrus

Travel Plans for Edinburgh Congress

Heredity-Environment and the Bent-Nose Rat

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The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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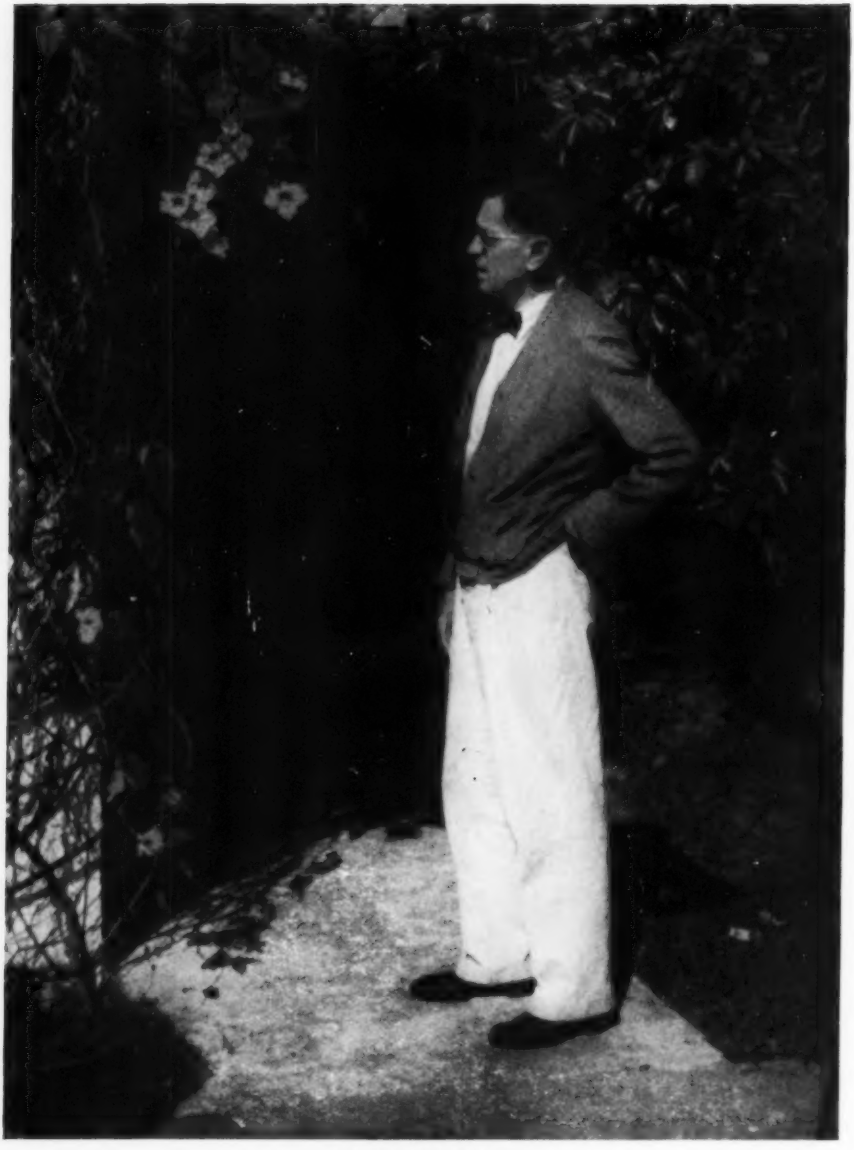

GUY N. COLLINS

Photographed at Coconut Grove, Florida, 1938

GUY N. COLLINS, PLANT EXPLORER AND GENETICIST

August 9, 1872—August 14, 1938

SO long as the mystery of death remains wholly unexplained, we stand beside the bier of a friend no whit wiser than were the men of the Stone Age. So it is with the age-old feeling of utter loneliness and an inability to see reason or order or anything else in the way of cause or desirability or necessity that I attempt to set down here a few memories of my old friend, Collins, who has just passed away.

Had I not known him so well and esteemed him so highly it would have been easier to have a perspective on his life and work. As it is, when I try to write of him emotion blurs the picture. Growing in my garden in Florida is the Collins avocado which he brought from Guatemala and which was one of the first to come from that country into ours. Selected from among its progeny came a superior variety also called for him, the Collinson which is widely grown in Florida today. For many years I have carried in my pocket, as a part of my hand lens, an attached needle which is so valuable an addition that it deserves to be an integral part of every hand lens. This is always associated in my mind with Collins rather than with Hillman, the inventor, because it came to me through Collins, whose enthusiastic appreciation of really useful and effective improvements was always delightful. His appreciation of such inventive approaches had the craftsman's ardor, because he devised himself, and aided others in devising, many improvements of equipment and methods of research. I seem to see Collins in the early days of botanical photography devising a clever arrangement to eliminate the shadows in his pictures of plants. He mounted the camera and the object to be photographed on a swinging stand and whirled the whole stand while the exposure was being made. He was one of the pioneers in advancing the idea that natural size photographs of botanical material were of unique value as illustrations and as records.

These are tangible objects which I can feel and handle. But I have as well a whole series of memories beginning with those that are faded and vague with time. They date from the early days of our friendship, when as a youth he accompanied O. F. Cook to the Dark Continent on an expedition designed to solve for the colored race some rather fundamental problems, such as the feasibility of transporting the American Negro to Liberia and establishing him there in jungle clearings. Three voyages to Liberia were made, the first on a small sailing ship, leaving New York in October, 1891, not reaching Monrovia until December.

I can follow him then to Puerto Rico, and I think of him when I see the magnificent photographs he took there of tropical fruits and flowers in those early days of America's awakening to the value of the tropics. No book on tropical vegetation has been so well illustrated as the one he and Cook wrote after their stay in the island in 1899, just after it had become a part of the United States, following the war with Spain.

It was this exploration of Puerto Rico that led to his writing a most excellent bulletin on the tropical mango which appeared at a time when the name of this great fruit aroused little more than a smile among Americans who knew of it only as a fruit which "should be eaten in the bathtub."

Of the several explorations he made

FOREST AND RIVER

Figure 1

Clearing in the tropical Liberian forest on the St. Paul river. From 1891 to 1896 Collins accompanied O. F. Cook on three expeditions to Liberia to study conditions for the Liberian Colonization Society, which was interested in sponsoring a "Back to Africa Movement" for American Negroes. A cabin was built on the St. Paul River to study the natives' living conditions and agricultural practices. This photograph made by Collins gives an excellent impression of the tropical forests which cover much of Equatorial Africa.

in Guatemala, Costa Rica and Mexico beginning in 1902, I know only through the accounts he gave us from time to time, from plants he brought back and from that first bulletin on the avacado in which he called our attention to the extensive use made in the highlands of Guatemala of the hard-shelled, long-season varieties of this important tree crop.

"Waxy" Maize

When I came back from one of my journeys Collins met me with a most enthusiastic account of a strange kind of corn sent by a missionary in China, and we obtained further information about it from that remarkable little woman, Dr. Yamei Kin, from whom we received all sorts of Chinese cultivated plants during the many years of her association with us as a collaborator of the Department of Agriculture. This corn had what Collins described as a "waxy endosperm," instead of the usual starchy kind, and this character of waxiness turned out to be one of those heritable characters of maize of which he later wrote so much. I am not sure that it did not start the series of papers which ran through many numbers of the JOURNAL OF HEREDITY over a long period of years. In the 1918 volume, page 154, he suggested the utility of such a collection of papers. In these words he emphasized the problem: "I would strongly urge that each of the maize

AT HOME IN THE JUNGLE

Figure 2

The Liberian expeditions (1891-96) carried on their studies of native customs and problems from the St. Paul River Station at Mt. Coffee. The cabin and its furnishings were built on the spot. Collins was twenty-three when this photograph was made.

growing countries of America make a canvass of the maize varieties growing within its boundaries. The work of discovering new and valuable characteristics is one in which a cooperative arrangement among the different maize growing countries of America would be especially applicable. Once an adaptation is observed and a discovery announced, it becomes available to all countries. * * * * I venture again to call attention to the necessity of looking for desirable adaptations rather than desirable varieties and to urge that no variety be overlooked simply because it appears insignificant and worthless. If a variety is growing under climatic conditions that are extreme in any particular, it is more than probable that the variety possesses valuable characteristics."

How often have these words returned to me when I have been tempted to pass by some nondescript ear of corn without collecting it as I have passed through a native bazaar somewhere in my own travels! I cannot say with certainty that this idea started the search for "adapted genes" and "superior germ plasm" which has been so strongly emphasized of late, but I am sure it has led to the creation of new and valuable varieties by combining characteristics found in plants introduced from far corners of the world.

Glimpses

But I can only give here a few glimpses of Collins and trust that they may interest those who come after us who may care to know what kind of a man it was who started all sorts of things that were new; things, which as the years pass, may seem more important than they now seem to be. To the friends who were talking with him only

yesterday these may seem trivial, but as I see biography, its charm consists mainly in such glimpses. But how difficult it is to give these pictures in word descriptions!

Collins was of all my friends one of the most open-minded. He might laugh at my enthusiastic description of some new vegetable or fruit, but he would always be willing to try it. When he returned from Africa he convulsed those of us who dined at the same table with him in the little boarding house on Wallach Place by his description of the "dumboy" made by the native Africans. He was the first to be interested in my account of the "Rijstaafel" (rice table) which I had learned to like in Java. It was Mrs. Collins who with his help contrived what I believe was the very first American edition of this famous and complicated dish.

His and my own passion for tasting new tropical fruits and nuts once got us into difficulty. We both felt so confident that the nuts of *Jatropa curcas* were always good to eat that we picked some from a tree in the Plant Introduction Garden here in Florida and ate them. We found them delicious but they prostrated both of us in a rather spectacular way which our friends never forget.

Writer and Traveller

Collins had a way of presenting the information he wanted to impart, which was very unusual in its clarity and directness. Whether he was describing for the *Geographic Magazine* a complicated game of counters played by the native Liberians, or merely giving us an account of how the Japanese Kudzu vine climbed to the top of his water tower in Maryland and covered it with an incredible mass of vegetation,—the result was the same. You saw what he wanted you to see and saw it clearly.

I never had the privilege of travelling with Collins; I know only from others what a delightful travelling companion he was, and that no discomfort or delay ever ruffled his composure or depressed his spirits. Most of my experiences with him were confined to meetings in Washington during office hours or in "The Mess" or at his home in Lanham, Maryland. And yet, among the very many friends of my Washington life his personality stood out so clearly that whenever in later years I thought of a visit to Washington I saw Collins in his swivel chair at his desk in the basement room of the "West Wing," ready to greet me with a smile and a joke and a question.

He seemed to me always a boy; a boy whose curiosity about everything had never been dulled by his contact with the round of activities, the human controversies, and the cramping environment of committee work in Washington. He liked to see and touch and examine a thing; just to read what someone had said about it did not interest him nearly so much. To a very unusual extent, even among scientists, he looked straight at things, and he let the facts be a guide to conclusions. He did not adopt his ideas as intellectual foster-children who must be protected in a cruel world regardless of their ultimate merits, whatever the factual odds against them.

Such a thing as an acrimonious dispute was hardly possible with him, for he seemed to realize instinctively what most of us never learn, that words are only crude symbols and that one's own smybols may be, and probably are, quite different from those of other people.

He walked with a free stride and his swing of the head was characteristic. His crop of dark hair showed no signs of growing thinner as he aged and I remember it as without a touch of grey. He was almost always with a pipe somewhere about his person and when I see him in my memory he is engaged in filling or lighting his pipe, picturesque operations, both of them.

A Garden in Maryland

The memories I would especially like to make live after him are connected with a little patch of corn which he grew near his home at Lanham, Maryland. Perhaps this is because I believe in the seclusion of a small garden and its free-

EXPLORING IN COSTA RICA

Figure 3

Beginning in 1899 Collins made many botanical explorations of tropical America. This photograph was made in 1903, on an expedition sent by the U. S. Department of Agriculture to study tropical agriculture in Central America. Later, when Collins' interest shifted to maize, he made many long journeys through Mexico and Guatemala to study maize in the habitat in which it is assumed to have arisen, and to look for related grasses which might have been the ancestors of this unique crop plant, or which might have given rise to it through hybridization.

dom from those incessant interruptions which an office always brings, and perhaps because for many years I had a little garden of my own in Maryland. It was in his garden that Collins showed me his brachytic dwarf maize plants, pointing out the characters which he found were heritable, such as waxiness of endosperm, striping, and many more. From data collected on corn plants grown in this unpretentious plot of ground came the mathematical proof of the first linkage to be discovered in maize, only a few months after Morgan and his pupils had announced the gene theory. This linkage was between the genes for waxy *vs.* starchy and for purple aleurone color *vs.* white aleurone, the material having been gathered in Collins' studies of the challenging new character from China. I recall one visit with peculiar pleasure. I had gone out to see some of the Peruvian maize with kernels four times the size of ordinary maize kernels but which unfortunately refused to fruit in the short season of Maryland. We spent the late hours of the summer afternoon among his plants and then retired to the porch to talk of the Mayan civilization with which his travels in Guatemala had made him familiar. Perhaps it is an idle thought

that had Collins been so situated that he could have spent more of his time in a garden of his own choosing and less between the hours of nine and four-thirty in an office of desks and clerks, with the necessary street car or automobile ride to town through crowded streets and another ride home through the same streets, he would have given us even more interesting ideas and discoveries than he did.

I like to think of my friend as exemplifying the idea that if you become really interested in a thing the whole world will seem to swing around that interest and it will take you far and wide over the surface of the earth.

Collins might also be taken as an example to show how frequently something that looks most uninteresting at first sight will turn out to be romantic and fascinating as you know more about it. To a boy in his teens hoeing corn on a hot summer day, could anything seem less likely to lead to travel and adventure than a study of the tassels and the silk of this tiresome crop? Yet the studies which Collins made of those very common objects led him into the secrets of the cell behaviour of the corn plant. Because maize is, next to man, one of the most widely hybridized organisms in the world, this similarity has made some of the discoveries in corn applicable to the study of human beings.

During his travels in Central America he showed not only that maize is a hybrid plant but that there is nowhere to be found a wild form: that maize, of all our crop plants is perhaps the only one which seems to be wholly dependent on man to perpetuate the species. Collins concluded that it must somehow have originated through the crossing of two wild grasses. What were these grasses, and where did they grow were questions that led to travels in Mexico and Guatemala, travels to discover if there were still living any of the original wild grasses which, by crossing, might have given rise to maize, one of man's greatest food plants. His researches led to all sorts of archaeological and geographical investigations. He studied the findings in the mummy wrappings of the Incas; and was much interested in reports of maize being cultivated prior to 1492 by primitive peoples in the mountains of Northern Burma and Tibet, as well as in China. He did not find proof of its being known in Asia before the discovery of America. His attempts to re-create this unique plant by hybridizing its nearest relatives never produced "synthetic maize," but they did open up a most interesting field of research which is being tilled by other workers.

In other words, his curiosity put him in touch with strange lands and long forgotten remains of ancient man, and carried him forward to the very borderland of knowledge where the keenest minds are striving to lift the veil which hides from our view the workings of the chromosomes and the genes which control the forces of heredity. Man has barely begun to glimpse the potentiality of these forces which are probably destined, more than any others, to control his behavior on this earth in the years ahead. I know of no other friend who has more fully appreciated and loved this world of romantic possibilities.

He was an early member of the American Breeders Association, which in 1913 became the American Genetic Association, and he was an occasional contributor to the columns of the JOURNAL OF HEREDITY. His style was so lucid that his articles were often appreciated by those who do not ordinarily read technical genetic discussions.

As a member of the Council since 1918 his sane advice and even-tempered consideration of the many difficult questions which have arisen in the conduct of the JOURNAL have helped materially in their solution. It would be difficult to find a more conciliatory and yet positive personality than Collins. His open-mindedness and ability to get to the bottom of a situation made him of unusual assistance in building up and maintaining the scientific standards of the JOURNAL.

We shall all miss keenly the generous loving soul whose appreciative outlook on life made him a host of warm friends wherever he went. —DAVID FAIRCHILD

THE NATURE AND ORIGIN OF FILTRABLE VIRUSES

Considered in Relation to the Bacteriophage and in the Light of the Theory of the Gene

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EDITOR'S NOTE: *Speculation, if it does not "carry across" and become accepted as fact, has an important place in advancing scientific discovery. The following most interesting attempt to correlate some of our knowledge regarding filterable viruses, bacteriophage and genes will doubtless create violent opposition in some quarters and other profound reactions elsewhere. Some of the observations and conclusions on which Dr. Lindegren bases his thesis are not universally accepted by all workers in the fields in question. At the risk of engendering heat as well as light, the editor has found considerable support for the view that Dr. Lindegren's expedition into the speculative hinterland of the gene and its non-corporeal neighbors (as far as accepted definitions of vital corporeality are concerned) may bring some very useful synthetic thinking back alive and kicking enough to be tested experimentally, and verified or quietly made an end to. This possibility is alluring enough so that the editor has gone forward hopefully, even though he fully realizes that much verbal fire and brimstone may descend upon him because he has opened his pages to something else besides the recording and codification of "demonstrated fact,"—whatever that may be!*

THE theory of the gene holds that the gene is the primary living particle and the basis for life in all living forms. The gene is a unique entity which is capable of self duplication in a chemical environment consisting of substances unlike itself. In addition it produces an enzyme through which it exerts a specific effect upon the environment. Alexander and Bridges[1] suggest that bacteriophage may consist of free genes; and they classify different degrees of complexity of living forms on the basis of the association of genes into gene strings and nuclei. Northrup[14] isolated a nucleoprotein with the properties of the bacteriophage and Stanley[16] isolated a protein with the properties of the tobacco mosaic virus. The constant association of nucleoproteins with the nucleus and particularly with the chromosomes has strengthened the view concerning the identity of genes and nucleoproteins. But most of the nucleoproteins of the nucleus are probably genically inert material which act to insulate the gene. In organized living beings (in contrast to filtrable viruses) the gene does not come into close contact with the external environment. The non-living cytoplasm acts as an intermediate environment and it is through action on the cytoplasm that the gene complex exerts its action on the external environment. Compared to the cytoplasm, the nuclear sap contains very little protein. When the nuclear membrane disintegrates during mitosis, the gene strings are insulated by a coating of chromatin which prevents them from coming into contact with the cytoplasm, and possibly restricts the production of enzymes by the genes during the process of separating the already divided chromosomes.

Wrinch[17] has suggested that the chromosome is composed of "homologous protamine molecules helically around a quasi-cylindrical surface." Belling[3] has

presented cytological data showing that each chromomere contains one central granule enclosed in a cavity formed by the nucleoprotein which makes up most of the chromosome. Bridges (unpublished) has further suggested that the Wrinch grating, which is continuous from one end of the chromosome to the other, is so constructed that new molecules of nucleoprotein can be inserted to permit simultaneous longitudinal division of the entire chromosome by invagination of the grating preceded by division of each of the genes included in the chromosome according to Belling's early concept. The enlargement of the vesicle surrounding each gene might be the controlling factor which would give the gene sufficient space to duplicate itself just before the invagination of the grating occurred.

Free Genes

After telophase much of the nucleoprotein envelope around each chromosome disappears and the nearly achromatic gene strings are imbedded in the dilute nuclear sap which is separated from the cytoplasm by the firm nuclear membrane. The writer suggests that the nucleoprotein envelope is thinned to permit a freer interchange of molecules between the genes and the nuclear sap. However, the genes remain trapped in their proper order in the gene string although the meshes are more permeable than at metaphase. One of the principal postulates of the writer's view is that the naked gene is incapable of restricting its division in a rich nutrient and conversely, that the function of the non-genic nucleoproteins in the envelope is to restrict the reproduction of the genes and to insure that this reproduction occurs simultaneously. The cytoplasm is a rich nutrient and if free genes were permitted access to it, competitive multiplication might occur so rapidly that a diseased condition would result. By restricting the reproduction of the genes, the non-genic nucleoproteins and the nuclear membrane make the establishment of a diverse population of cooperating genes in one nucleus a possibility. The bacteriophage and other filtrable viruses probably multiply without restraint when they come in contact with a favorable cytoplasmic substrate because they do not have a restrictive non-genic nucleoprotein envelope. Nucleoprotein envelopes are conceived to be necessary, not only to maintain genic balance, but in addition, to prevent the escape of genes from their nuclear isolation.

Only the exceptional free gene, however, would be capable of attaining virulence as a virus. If this were not so, the enormous frequency with which autodigestion of tissue occurs in an organism would result in producing numerous autogenous parasites. Probably most organisms are immunized to their own genes[12] and only the rarer genes which act as poor antigens can attain virulence. The antibodies capable of inactivating the genes are probably confined to the cytoplasm and are probably unable to pass through the nuclear membrane to reach the metabolic genes in the anergic (resting) nucleus. Similarly the dense non-genic nucleoprotein grating probably prevents the gene-antibodies from acting on the genes in a metaphase chromosome. Therefore, the nuclear membrane and nucleoprotein envelope serve the double purpose of protecting the cytoplasm against the antagonistic genes as well as protecting the genes against the antagonistic cytoplasm. Intimate contact of cytoplasm and gene will destroy either one or the other. The mutual cooperation of genes and cytoplasm in the construction of a living organism is the direct result of the controlling influence of the interposed membranes. The most characteristic feature of the cytoplasm is its ability to produce antibodies. It is the unique factor which has made the association of many genes into one complex possible. The antibody-producing mechanism protects the food stored in the cytoplasm from destruction by genes accidentally freed from the nucleus.

According to the views already expressed, any action which seriously disturbs the structure of the cytoplasm will inactivate the gene complex. Irreversible heat coagulation of the cytoplasm may

still leave the genes potentially viable. However, since they are no longer presented with the raw materials with which to reproduce themselves or to produce the enzymes through which they express themselves, the organism, not the genes, dies. Heat coagulation has extremely deleterious effects on the cytoplasm of bacteria and fungi as well as on the cytoplasm of higher plants and animals. Chemical coagulation of the cytoplasm of higher plants is as serious as heat coagulation. They are invariably killed by immersion in absolute alcohol. This is probably because their cytoplasmic proteins are irreversibly coagulated by alcohol. Bacteria and fungi are different in that it is possible to dehydrate their cytoplasms in such a manner that when water is subsequently added, the coagulated cytoplasm again takes up water and returns to its original uncoagulated condition. The zygospores of some Mucors, as Koehler showed,[10] can be kept in concentrated alcohol for months and are completely viable when placed in alcohol free media. Absolute alcohol does not kill all bacteria because it dehydrates them before the proteins are coagulated. But 70% alcohol is a fairly good bactericide because this mixture irreversibly coagulates the bacterial cytoplasm. If the cytoplasm is reversibly deprived of metabolic water by freezing, (bacteria and fungi survive in liquid air) by spore formation, or by treatment with absolute alcohol, when the cytoplasm is again dispersed by liquid water the genes are able to carry on where they left off. Some of the spores of fungi and bacteria (in which the cytoplasm is apparently in a condition not coagulable at moderate temperatures) can be subjected to temperatures which would irreversibly coagulate vegetative forms. Staphylococci dehydrated by absolute alcohol can be subjected to a temperature of 120°C. for one hour, but when cooled to room temperature and suspended in alcohol-free media, they are able to continue their life processes.[13] Their animation was suspended, but their viability was unimpaired. Suspended in pure water they can be killed by exposure to a temperature of 60°C. for one minute.

How Define "Living"?

Earlier physiological definitions of living beings were not based on the gene theory, and were incorrectly concerned with the physiological characteristics of the non-living cytoplasm rather than with the relatively minute fundamental genic components. In studying irritability and metabolism, the gross changes in the cytoplasm are studied rather than the regulatory changes originating in the genes. Thus the older definitions concerning irritability, capacity to respire, etc., all involved characteristics of the cytoplasm rather than characteristics of the gene itself. These older definitions do not apply to free genes stripped of cytoplasm, and a new and more fundamental set of criteria must be used to embrace all living forms. Alexander and Bridges have established the necessary criteria and have pointed out that there is no intermediate condition between living and non-living, but different types of living forms may be differentiated by the degree of complexity which characterizes the association of the genes. The incorrect view that the bacteriophage and other filtrable viruses are non-living is based on data indicating (1) that they are more resistant to many agents, especially chemicals, than other admittedly living forms (Bronfenbrenner[5]), (2) that attempts to measure respiration (bacteriophgae) have not met with success,[5] (3) that they may not be particulate[5] (i.e. may be absorbed on other particles much larger than themselves), (4) that they may be crystalline compounds, and (5) that they may be enzymes capable of reproducing themselves (Zordet[4]).

(1) From the definition of living substances established by Alexander and Bridges,[1] it follows that tolerance to physical and chemical agents is not a valid criterion for distinguishing between living and non-living. The inability of the higher organisms to withstand environmental extremes is probably due to the instability of certain nonliving, cytoplasmic components of the cell rather than to the instability of the living particles.

(2) The fact that it has not been possible to observe measurable respiration in the case of bacteriophage has led to the view that it is non-living. However, this is not a valid criterion. In higher forms the ratio of cytoplasm to gene is enormous and it is possible that all the oxidative changes classed as respiration occur in the cytoplasm and are only regulated by the enzymes which the genes produce. In the cytoplasm, substances obtained from the external environment are altered and modified by the enzymes produced by the genes; only a small amount of the material reaches the gene itself. If the gene were free or enveloped by a highly permeable membrane and supplied with the materials necessary for its duplication and production of its enzymes, it would not have to carry on an extensive alteration of substances, with the resulting respiration to maintain an active existence. Such a condition would obtain if the gene were parasitic in the cytoplasm of an organism which supplied it with the necessary materials already elaborated into the form of highly developed "precursors" postulated by Krueger.[11] A filtrate containing bacteriophage might show no gaseous interchange with the atmosphere and still contain particles of living substances. There is no *a priori* reason why the activity (multiplication) of a simple living substance could not be suspended indefinitely. Attempts to suspend the life processes of higher forms usually fail, not because the genes die, but because some of the highly active metabolites of the cytoplasm disintegrate.

(3 and 4) It has been possible to show by crossing-over that the genes in higher forms are particulate, that is, definitely localized in space. On the basis of the molecular theory, any chemical substance must be particulate. All living substances are not necessarily organized to the same degree of complexity. The heirarchy of different living organizations has been treated by Alexander and Bridges. There is no reason for limiting the definition of living substances with regard to the size, solubility, or crystallizability of the living particle.

(5) A gene is an autocatalytic living particle which produces an enzyme. Therefore, the view that the bacteriophage is an autocatalytic enzyme, is in agreement with the view that it is living.

Those who hold that bacteriophage is living, differ in their views concerning its origin. D'Herelle's view that the bacteriophage, like other forms of life is produced by simple multiplication of preexisting bacteriophage particles, differs from that of Bail[2] who believes the bacteriophage to be a freed gene or chromosome fragment. It has been suggested above that if a gene were freed of most of its non-genic nucleoprotein envelope, it might be capable of invading a cell similar to that from which it was derived. If the nucleoprotein is not dense enough to restrict the division of the gene, multiplication could occur in the cytoplasm. Bacteria and fungi usually produce disease by intercellular growth, but filtrable viruses and Rickettsia grow within the "living" cytoplasm of the host. Many filtrable virus and Rickettsia diseases of plants and animals are transmitted by insects. These insects ingest and digest cells containing nuclei. If digestion of mammalian cells can occur without destroying the genes in the digested cells, it may be possible that on rare occasions the cell digest can contain a virulent free gene or chromosome capable of parasitizing the insect because it is so "weak" antigenically that the insect is incapable of becoming immune. After a long infestation of the insect, the mammalian gene might mutate until it is antigenically different from the original mammalian gene. When reintroduced into the animal from which the gene originated, these free genes might be even more capable parasites. However, this must occur only extremely rarely in the course of evolution, for although there is much experimental evidence to support the theory that bacteriophage can originate from "bacteria alone," there is no experimental evidence even suggesting that other filtrable viruses arise spontaneously.

Otto and Munter[15] have reported the production of bacteriophage from bacteria. The first step in their process is to hold the bacteria at about 60°C. This probably irreversibly coagulates the bacterial cytoplasm without harming the genes. Although autodigestion may serve to immunize higher plants and animals against most of their own genes, in free living cells like bacteria, the opportunity for auto-immunization is at a minimum. This may explain why bacteria are more seriously plagued by a greater diversity of filtrable virus diseases than any other living forms. However, when they are left in contact with a particular bacteriophage they soon become immune. The chief difficulty in accepting data concerning the spontaneous origin of bacteriophage is d'Herelle's statement that the experiments are invalid because "infected" bacteria may have been present in the original culture. The presence of even very few extremely resistant infected "carriers" must be critically excluded in any such experiment.

Northrup[14] has isolated a nucleoprotein with the properties of the bacteriophage. Dr. A. P. Krueger suggested to the writer that this compound may differ from the non-genic nucleoprotein envelope of the gene in the special orientation of the fundamental nucleoprotein molecules. The total molecular weight of Northrup's bacteriophage corresponds in size with the particle which Belling observed at the center of the destained chromosome. The "precursor" described by Krueger could be an aggregate of nucleoprotein molecules which might be fully prepared to attach to the reproducing bacteriophage in a manner similar to that suggested by Alexander and Bridges in their hypothesis concerning gene reproduction. The inconsistency of X-ray experiments determining the particle size of bacteriophage and genes may mean that only a small fraction of the nucleoprotein aggregate is concerned in the manufacture of the enzyme by which the gene expresses itself and it is only "hits" in this particular region which prevent this activity from being carried on. "Hits" in portions of the genic aggregate of nucleoprotein molecules distal to the essential spatial arrangement may not inhibit the production of the gene's enzyme although it might interfere with reproduction of the gene.

Variations in filtration experiments might be due to the possibility that mechanical disturbances could separate the gene into various smaller "precursors" which reattach after filtration.

Summary

The cooperation of genes and cytoplasm may depend on two restrictive devices: (1) the chromatin envelope of the gene which prevents contact of gene and cytoplasm at mitosis and (2) the nuclear membrane which prevents contact of the more or less exposed, energic (resting stage) genes and the cytoplasm. Free genes and cytoplasm may be antagonistic because (1) the cytoplasm may contain gene-inactivating antibodies and (2) genes in direct contact with the cytoplasm (if not inactivated by antibodies) might divide without restraint and destroy the cytoplasm together with the food stored in it. The interposed chromosomal and nuclear membranes make it possible for the genes to transform and direct the energy supplied by the cytoplasm.

Acknowledgment — The writer is grateful to Dr. A. P. Krueger for his criticism and revision of the manuscript. His concept of the gene as an aggregate of nucleoprotein molecules oriented to produce a specific spatial arrangement which acts as the "enzyme-factory," according to Bridges' concept, seems to reconcile many of the earlier inconsistencies.

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RECENT ADVANCES IN THE CHEMISTRY OF CHROMOSOMES

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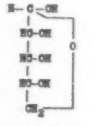
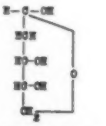
THE huge chromosomes in the salivary glands of the fruit fly have received much attention from cytologists and geneticists since their "genetic discovery" by Painter four years ago. Only recently, however, has any biochemical work been attempted using these chromosomes as material. Such is the size of these chromosomes that micro-biochemical tests may be made, and the results are of great enough magnitude so that they may actually be studied on the chromosome itself, instead of en masse. In recent years some very important results have been obtained by microbiochemical analysis of other materials, but it remained for the genetic significance of the salivary chromosomes to be established before a chemical approach could show definite relations between genes and chemical structure.

The results of macrochemical work to 1931 are well summarized by Levene and Bass in the book "Nucleic Acids"[7]. By that time, both plant and animal nucleic acid (the characteristic acid of nuclear material) had been isolated and all of its components were definitely known. Its structure was not absolutely certain, but was bracketed within rather narrow limits. Yeast nucleic acid and thymus gland nucleic acid are recognized as the typical acids characteristic of plants and animals, respectively; and it has even been suggested[1], from a careful study of nucleic acids from a variety of sources, that perhaps only these two compounds are to be found in organisms, though other workers doubt that the matter is as simple as that.

From the results of these macrochemical analyses, it is definitely known that all nucleic acid is made up of combinations of three groups of fairly simple compounds. The simplest of these groups is the inorganic phosphate acid group PO_4. Another group in the makeup is the pentose-carbohydrate d-ribose or its derivative d-ribodesose. Its structural formula and that of its derivative are given below:



d-Riboe



d-2-Ribodesose

The third group of compounds consists

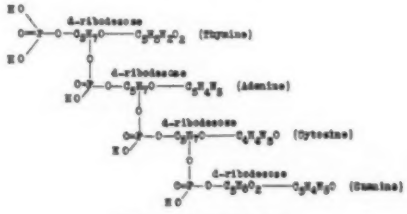
of members of the purine and pyrimidine groups, the pyrimidine group being the simpler. The pyrimidines found in nucleic acid are thus diagrammed:

Adenine Guanine

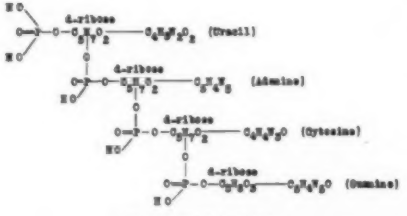
Three members of the purine group found in nucleic acid are considered to have the following structure:

Cytosine Uracil Thymine

Structurally, nucleic acid is made up of four linked chains. Each chain consists of a phosphate acid group linked to a pentose group (D-ribose or ribodesose) which, in turn, is linked with a purine or pyrimidine group. The four chains are linked together through the phosphoric acid. The two types of nucleic acid (thymus gland and yeast) are very similar in their structure and are generally diagrammed as follows:



Thymus Nucleic Acid



Yeast Nucleic Acid
(after Levene and Bass)

It may be noted by comparing the components of these two acids that there is very little difference in their make-up:

Yeast Nucleic Acid		*Thymonucleic Acid*
adenine		adenine
guanine		guanine
cytosine		cytosine
uracil	(different)	thymine
d-ribose	(different)	d-ribodesose
phosphoric acid		phosphoric acid

Using these results of former macrochemical analysis, T. Caspersson, in 1935-36, reported work in which he detected nucleic acid by two methods in testicular cell nuclei[3,6]. Utilizing a method of characteristic wave-band absorption in the ultra-violet range, he was able definitely to locate nucleic acid in microscopic amounts. He confirmed this by using chemical methods for the microprecipitation of nucleic acid. The reagents in this case were mixtures of lanthanum salts with proteolytic enzyme. The enzyme digested, or put into solution, any proteins associated with the nucleic acid, while the lanthanum salts precipitated the nucleic acid. Thus he obtained a precipitation of nucleic acid without interference from proteins. The results of these investigations showed several interesting and significant facts: (1) Nucleic acid is not solid in the testicular chromosomes. (2) Resting cell nuclei have haphazard distribution of nucleic acid. (3) Active metabolism (as in a functioning gland) tends toward extreme variation in nucleic acid content. (4) In connection with mitotic division, (a) nucleic acid content in the cell rises just before visible mitosis, (b) nucleic acid is localized in the chromosomes during early prophase and is intimately mixed with proteins, and (c) nucleic acid content decreases immediately after division.

Caspersson recognized the value of the giant chromosomes in the salivary glands of Drosophila and Chironomus larvae in this work. Applying the same lanthanum-salt-proteolytic-enzyme mixture to these chromosomes, he obtained[2,5] precipitation of nucleic acid and found the resulting precipitation pattern the same as the usual pattern obtained with aceto-carmine stains. Thus, the discs as

seen in the normal salivary chromosome precipitation were found to be rich in nucleic acid, while the clearer spaces between the discs contain little or no nucleic acid. Under continued digestion with the reagent, the disc structure is loosened and occasionally splits up into thin platelets showing the presence of some protein in the discs. In this way it was found that the two types of segments, *viz.* those rich in nucleic acid and those poor in nucleic acid, "contain protein substances but that the segments rich in nucleic acid show, after digestion, that they possess a highly organized structure."

Caspersson and Schultz have very recently[4] shown a close relationship between nucleic acid snythesis in the chromosomes and gene reproduction (as in mitosis or meiosis). They state:

> It seems hence that the unique structure conditioning activity and self-reproduction, possibly by successive polymerization and depolymerization, may depend on the nucleic acid portion of the molecule. It may be that the property of a protein which allows it to reproduce itself is its ability to synthesize nucleic acid.

They also suggest a relationship with the interesting case of "other self-reproducing molecules — the viruses and the bacteriophage — all of which have been shown to contain nucleic acid."

Miss D. M. Wrinch, of Oxford University, postulated[8] a very plausible chemical construction for the chromosomes, with this work as experimental data, and by reference to mitotic behavior, "requirements of Genetics," and identity in the protein constituent of the banded structure. She locates the genic nucleoprotein complex, claiming that certain unknown amino acid arrangements in the protein are characteristic for the gene and that these nucleoproteins (possible genes) form linear bands in the chromosome. She also postulates nucleic acid attracted in rings around the protein chains, if the pH of the chain is alkaline. Acid proteins supposedly collect no nucleic acid. Therefore, a negative test for nucleic acid would mean an acid pH, not lack of genic material. The bands also would then be due to an "alternation of basic and non-basic units in the protein pattern as providing a picture of the chemical situation."

Thus we see that macrochemical analysis has given us the basis for the microdetection of nucleic acid, by the disclosure of its structure and chemical makeup, and by the discovery of tests for nucleic acid applicable on a micro-scale.

Caspersson and others adapted these tests to micro-work, and thereby were able to demonstrate: (1) the change in quantity of nucleic acid during resting metabolism and mitosis, (2) the shifting in location of nucleic acid during resting metabolism and mitosis, and (3) the cytological location of nucleic acid in chromosomes, particularly the salivary chromosomes of Drosophila and Chironomus larvae. Using the results of this work in connection with other known facts, Miss Wrinch has postulated a plausible theory for the chemical makeup of chromosomes, holding that the banded structure is a secondary "by-product" of acid-base relationships in the proteins of the chromosomes.

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EFFECTS OF REPRODUCTION BY NUCELLAR EMBRYONY ON CLONAL CHARACTERISTICS IN CITRUS

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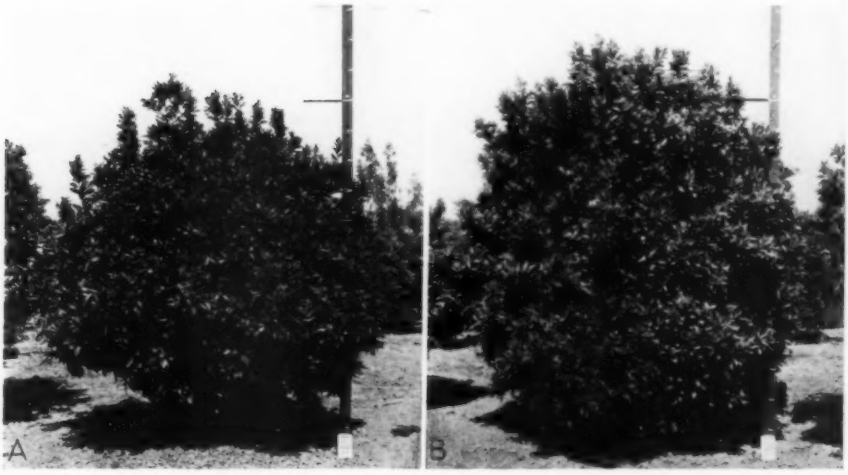

TWO CLONES OF THE PAPER RIND ORANGE

Figure 4

Invigoration of the Paper Rind orange variety caused by nucellar embryony. The two clones appear to be genetically identical and the trees are of the same age, seven years from planting. *A*—Tre of the standard (old) clone. *B*—Tree of the clone obtained by nucellar embryony. The black line marks the eight-foot mark on the scale.

SEVERAL investigators[1-5] have reported that the asexual seed reproduction of Citrus, by the formation of embryos from cells of the nucellus, results in a marked and persistent rejuvenation, or vegetative invigoration. When for example young trees of an old clone of sweet orange are compared with trees of the same age belonging to a young clone of the same variety, propagated from a recently produced nucellar seedling of the old clone, rather striking differences appear. Frost[5] says:

> The evidence indicates that the trunk of a thorny seedling (or tree budded from a young seedling), and the proximal portion of its main branches, retain for a long time (probably indefinitely), the ability to produce thorny shoots, which when used as bud sticks will produce thorny trees. On the other hand, the shoots far from the trunk, and especially, it appears, the uppermost ones, show a tendency to progressive reduction of thorniness and of the ability to transmit thorniness in budding. Trees budded from young seedlings of apogamic origin do not bloom so young as trees budded directly from ordinary orchard trees of the same variety. As seedling thorniness declines in successive propagations by budding, the trees tend to bloom younger.

The thorniness of seedlings is a matter of common knowledge with citrus growers and has been reported by workers in several countries: Webber[6] found that Florida growers believed that thorniness had been eliminated from old clonal varieties by repeated selection of

budwood comparatively free from thorns, but stated his belief that the disappearance of thorns is due rather to a weaker tendency to development of thorns on old trees.

It appears that nucellar-seedling propagation in Citrus brings about a marked invigoration which, in contrast to the less pronounced invigoration produced by ordinary nursery propagation, is definitely propagable and seems to persist for a more extended period. Ensign[1] concluded "Rejuvenation seems to be effected equally well through apogamous and through genetic reproduction" in Citrus seedlings, and points out that Child[7] has found this to be true in other organisms; it is possible, however, that all the seedlings which Ensign studied were of nucellar origin. Swingle[2,3] and Tanaka[4] have discussed the invigoration produced by nucellar embryony in Citrus varieties and suggested its possible horticultural significance. We have recently indicated an application of this sort.[8]

Other workers have questioned whether such observations as the above are actually due to true "rejuvenescence," and have held that the observations can be explained in other ways. It is the purpose of this paper to place on record some observations on the effects which propagation by nucellar embryony seems to have produced on the characteristics of an old and well known clonal variety of Citrus. These it is hoped will be accepted as experimental evidence regarding a moot matter of considerable importance.

Experimental Materials

Through the courtesy of H. B. Frost of the Citrus Experiment Station, Riverside, California, budwood was obtained, in the fall of 1927, from the Paper Rind (St. Michael) orange tree used as seed parent in one of his crosses, and also from a tree budded from a nucellar seedling from this tree. Cytological examination by Frost had shown that the chromosome numbers of the two were identical. Carefully selected and rogued sweet orange seedlings, believed to be all of nucellar origin, were budded with buds taken from the two trees mentioned and the trees grown in the nursery until the spring of 1929. At that time the two largest and most uniform trees of each lot were transplanted to an orchard row where they were spaced 12 feet apart. Limitations of space precluded the use of larger numbers of trees. The trees all received the same care and the members of each pair have from the beginning remained remarkably uniform. The data here reported represent the average for each pair of trees, as recorded in May, 1936.

We have, therefore, in these two pairs of trees the comparison of the Paper Rind orange variety before and after reproduction by nucellar embryony — that is, of an old and a young clone of this variety — the age of the trees compared being identical and the growth conditions substantially uniform.

Results

Some of the results of the comparison are shown in Table I and Figure 4.

The invigoration resulting from reproduction by nucellar embryony is strikingly evident in both area of trunk cross-section and volume of top. The growth habit of the young clone is decidedly more upright and the branches and shoots, particularly in the lower part of the trees, much thornier. The earlier and heavier bearing of the old clone is

TABLE I. Characteristics of Paper Rind orange tree and fruit before and after nucellar-seedling reproduction.

	Area of trunk (cross-section above bud union) sq. cm.	Volume of top (as measured by fumigation tent) cu. ft.	Crop for 1935-36 season, lbs. (per tree)	Average seed content. Sample of 100 fruits.
Old clone	76.6	491.5	101.1	15.25
Young clone	105.9	1052.5	53.2	1.43

also evident; with twice the top volume the young clone produced, in the season of 1935-36, only half as much fruit. The trees of the old clone came into bearing two years earlier.

The difference in seed content of the fruit is even more pronounced. The highest seed content for the old clone was 27, and no fruits were seedless; the highest for the young clone was 8, and 41 fruits were seedless. Frost[9] reports somewhat similar effects in trees grown at Riverside.

Conclusions

A comparison of the Paper Rind orange variety before and after asexual seedling reproduction has shown marked differences between the old and young clones, which obviously resulted from nucellar embryony. The major differences noted are greater vigor, more upright habit of growth, thorniness and lower seed content in the young clone, and earlier and heavier bearing in the old clone.

It is concluded that by resorting to propagation by nucellar embryony the possibility exists of invigorating polyembryonic Citrus clones and of reducing the seed content of the fruit. In our opinion these possibilities may have important horticultural applications. If, as the weight of evidence indicates,[10] the premature decline of lemon trees of the Eureka variety, now so wide-spread in certain localities in California, is caused by lack of scion vigor, asexual seed reproduction of the variety may provide a solution for this problem. Trees which are the result of asexual seedling reproduction are being grown to determine the validity of this conclusion.

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A NOTE ON EMBRYO-REJUVENATION

THE accompanying articles deal with phenomena which, interesting in themselves, may have wider implications for genetics than has been generally realized. The peculiar form of asexual reproduction which is found in Citrus has already been applied in a practical way on a considerable scale. Its theoretical implications on the problem of "genetics of development" are just beginning to be appreciated. A brief résumé of what goes on in Citrus embryology may not be amiss. When an orange flower is pollinated, two distinct trains of events may be set in motion. (1) Fertilization results in the formation of a zygote, a true hybrid between the parents, which proceeds to develop in the "conventional" manner. (2) Side by side with this development, stimulated by the formation of the hybrid embryo, maternal tissue from the surrounding nucellus grows into the embryo sac and forms secondary embryos which then proceed to develop into functional embryos just as the associated zygotic embryo does.

Opening an orange seed one finds almost always several embryos usually varying in size. One may be a true embryo but it is not by

any means the largest. All of these embryos, true and false, show a pair of cotyledons, a plumule and a radicle.

The nucellar embryos are normal in their chromosomal constitution just as a bud is normal, since they arise from diploid somatic tissue. The developmental behavior of these nucellar embryos is so radically changed that they represent a *very special* form of asexual reproduction, for they *grow exactly like true zygotic seedlings,* not like buds. Ordinarily a terminal bud, or an axiliary bud, carries on from the point where it is and forms adult organs, flowers, leaves, etc. Cuttings from such tissue produce a lateral root system, but no taproot, as does a seedling. The nucellar bud developing in the embryo sac forms a normal embryo with cotyledons, plumule and radicle which on germination develops a whole galaxy of special morphologic units,—epicotyl, intermediate leaves, hypocotyl, tap-root, etc., exactly like a true embryo. That the effect of neophyosis is not transitory is shown by the observation that nucellar clones may be hardier than the parent zygotic clone, as in the case of the Silverhill Satsuma. This nucellar variety survived when the parent variety froze (SWINGLE and ROBINSON, Mimeographed Circular, Bur. Plant Ind., Jan. 3, 1931). Buds from commercial Citrus varieties produce virtually thornless trees, but nucellar embryos from such a variety may be just as thorny, or even more thorny, than the zygotic seedling with which they are associated.

This phenomenon of rejuvenation or "neophyosis" has obvious practical implications, and its genetic importance is great because of the problem it poses of what the mechanism is which is able to set back the developmental procss to a fresh start. All of the differentiation evolved in the growth to maturity or even senescence is cancelled and the bud begins over again and repeats its previous development *as an embryo, with the specialized tissues and organs of an embryo.*

The history of the discovery and of the development of this phenomenon is of especial interest if it turns out to have the wide implications which it very well may. In 1878 Strasburger traced the development of supernumerary embryos in Citrus seeds and established the fact that they arose from maternal tissue—the nucellus. He traced the train of events whereby nucellar buds penetrated into the embryo sac and grew there into embryos, often small and malformed because of mechanical crowding, but capable of developing into viable seedlings with true cotyledons, a tap-root, etc. These supernumerary embryos were thus proved to arise by a special form of asexual reproduction. The next step was made by Walter T. Swingle, who found that in zygotic hybrids of the ordinary orange (*Citrus sinensis*) and the trifoliate orange (*Poncirus trifoliata*), the hybrid seedlings develop trifoliate leaves only a few weeks after germinating, whereas the supernumerary embryos which developed from orange tissue growing into the embryo sac, could readily be distinguished because they had simple primary leaves. (*Yearbook,* U. S. Department of Agriculture for 1897, p. 400). This made it possible to distinguish definitely the nucellar bud seedlings from the true hybrid embryos even when the seedlings were still very small.

This discovery was soon taken practical advantage of to propagate cultivated varieties of Citrus. The method owed its usefulness to the fact that the nucellar seedlings were far superior to rooted cuttings as orchard stock, since the seedlings had a tap-root whereas the cuttings only developed a secondary root system.

Furthermore, the true hybrids turned out to be of considerable value and started a long investigation of experimental hybrids in Citrus. These Citrus × Poncirus hybrids were called Citranges, and several varieties of these have become of definite commercial importance. They had another interesting property in that they were ovule-sterile. That is, pollinated with any kind of Citrus pollen they developed seeds which contain exclusively nucellar bud embryos. This gave a rapid and convenient method of asexually propagating Citranges from seed. These nucellar bud seedlings of F_1 hybrids were as alike as identical twins and obviated the slow, cumbersome procedures of making cuttings or budding.

Swingle later developed the theoretical implications of this phenomenon and expressed the view that it must be due to the influence of the embryo sac, a maternal organ, on the embryo proper in the early stages of ontogenetic development. (*Mem. Hort. Soc. New York* 3:19-21, 1927.) This theory is difficult to attack experimentally, but the observed facts seem to conform perfectly to such an explanation. The concept that the embryo sac apparatus, which consists of a bewildering complex of nuclei, protoplasm, and tissues belonging to different generations existing side by side, represents a powerful morphogenetic field of force, comparable perhaps to the electrical stresses surrounding the pole of an electromagnet, is most interesting. It would seem to be a challenging point of departure for experimentally approaching the genetics of development, whereby we hope eventually to learn how genes in the chromosomes of the fertilized egg somehow guide the complex processes of growth and differentiation so that they culminate in the production of a functional adult organism. The fact that the nucellar bud, in the "biological field" of the embryo sac, has impressed on it a return to the embryonic stage of development was first pointed out by Swingle a year later at the annual meeting of the National Academy of Sciences (April 24, 1928), where he recognized the thorniness and other characters of the nucellar embryos as a juvenile characteristic.

At the 1932 Genetic Congress Swingle further developed the concept that neophyosis,

a term he there suggested for the first time, was truly a rejuvenating phenomenon. A return of juvenile characters was much more marked in old varieties, which had been propagated asexually for a long period, than in young varieties which had only grown a few years from the original seedling. Thus, the young variety, the Rusk citrange, recently produced from an F_1 hybrid, did not show striking evidences of nucellar rejuvenescence. Older varieties, such as the Washington navel orange, showed much more striking changes. It was suggested that the genes which actually control the early development of the normal embryo, as well as the development of the nucellar bud, may be lodged in the nuclei of the embryo sac, — not in the embryo itself. (*Proc. VI Int. Gen. Cong.* 2:196-7. 1932.) Later Swingle summed up his explanation of neophyosis in the following words:

"The embryo sac is frequently invaded by few-celled buds of nucellar tissue which in developing recapitulate very nearly the normal morphological structures of the true embryo—cotyledons, epicotyl, and intermediate leaves, hypocotyl, collar, tap and primary lateral roots Recently originated ovary-sterile hybrids reproduce almost exactly the original F_1 seedling but old cultivated varieties long propagated vegetatively usually exhibit marked rejuvenescence and probably revert approximately to their original youthful state. This unexplained recapitulation of embryonic and juvenile morphology may be due to hormones secreted in the embryo sac apparatus that force into activity, in the nucellar bud cells, genes governing embryonic ontogeny. The accompanying neophyosis or rejuvenescent action is possibly a by-product of these powerful influences which in forcing cells of old, more or less senescent, tissue to produce juvenile structures may, at the same time, cause them to take on the physiological activities appropriate to young and vigorous plants." (Advance abstract of Physiological Section, Botanical Society of America, Dec. 28-30, 1932.)

* * *

"Such false seedlings recapitulate in very striking manner the morphological structure of true seedlings, showing cotyledons, epicotyl, intermediate leaves, thorny twigs, hypocotyl, collar, tap root, and primary lateral roots, in spite of the fact that such false seedlings develop from nucellar tissue of the mother plant that has already passed through the seedling stage of ontogeny. . . . . .

"It is also difficult to avoid the conclusion that the extraordinary recapitulation of a stage in ontogeny already passed by the generation which furnishes the nucellar buds is impressed upon these buds by one or more of the generations that occur in the embryo sac and doubtless the striking rejuvenation of old varieties of Citrus is also a manifestation of the powerful morphogenetic influences that have their locus in the embryo sac apparatus of higher plants and perhaps exerted through hormone-like substances."

(SWINGLE, WALTER T. Metaxenia and neophyosis, two forms of morphogenetic influence exerted by the generation-complex of the embryo sac apparatus in higher plants. In a multigraphed summary to accompany the program of the Annual Washington Meeting of the National Academy of Sciences, April 24-26, 1933, pp. 27, 28.)

This is the background of the articles by Hodgson and by Frost which record evidence confirming Swingle's reports on neophyosis. If such influences as Swingle has postulated are in fact the guiding agency in early embryonic development, it will be a most important step in understanding ontogenetic processes. It will be an almost fatal blow to the somewhat naive proposal that we shall eventually perpetuate the human species by "ectogenetic" babies, raised not *on* a bottle, but *in* one, from the one celled stage. For it will reemphasize the perhaps obvious fact that development is not exclusively a process controlled by the fertilized egg, but is a cooperative interaction between the egg and the maternal tissues. The "egg environment" becomes an extremely complex affair, especially perhaps in plants and mammals, where interaction between mother and embryo during embryonic stages are considerably prolonged. Very broad parallels between animal and plants will hardly be found, though some curious interrelations may exist. Thus in plants, grafts and buds generally "take" within a considerable range of genetic types, while in mammalian tissue, grafts tend to be highly specific. In the diptera such tissue-specificity is evidently not developed, because viable inter-species grafts have been made. In mice, for instance, grafts within an inbred strain are positive, but inter-strain grafts generally fail. In spite of this high tissue specificity for grafts, embryonal development in mammals proceeds in general without the clash of genes which tissue transfer experiments might lead us to expect since the fetus may be very different in genic make-up from the mother.

We would expect to find confirmation of such a revolutionary concept in other fields. Often we only find what we look for, so if wide confirmation is still lacking that may only mean we have not looked, which hardly permanently disposes of the matter. Curiously enough, discovery by Swingle of metaxenia, also in the field, shall we say of paragenetics, fits well into the picture. In 1933 he noted the interrelations between metaxenia discovered in the date palm (*Journal of Heredity* 19:257-278, 1928), and neophyosis in Citrus. In the one case, hybrid tissue of the embryo or endosperm has a direct effect on the maternal tissue surrounding it. The other situation is the reverse of this, whereby the maternal tissue of the embryo sac profoundly effects the development of the embryo proper. Here we

have also conclusive evidence of interrelated reactions between maternal and embryonic tissues. Development begins to become not an all-or-none reaction in a favorable environment. Rather it is one more example of the principle of mutual dependence, with which biology abounds. The implications of the situations in Citrus and date palm may already be beginning to have wider ramifications. Thus in the September issue of the JOURNAL the recent discovery was discussed, that sheep-goat hybrids survive for about half of the normal period of embryonic growth and then die. It is pure speculation to wonder whether any metaxenial or neophyotic effects might be found in such a situation, but such a possibility is not inconceivable. If such influences are in fact as profound as the Citrus situation suggests we might expect to get definite differences in reciprocal crosses, differences which might determine the life or death of the embryo. It is possible that evidence of metaxenia in mammals may exist in the widely held belief that mules are superior to hinnies. A compelling reason for preferring the cross of the jackass and the mare is economic, — that the jackass is of less value than the horse and that mares are more useful *per se*, and easier to obtain, than jennies. But there seems to be more to the matter than that, for there is a wide-spread belief that hinnies are "harder to produce and are smaller and less useful than the mule." (Gay). If this be fact, and not rationalization from the economics of the situation, a strictly genetic explanation would have to put a rather heavy load on the *X* and *Y* chromosomes as furnishing the only consistent difference in the reciprocal cross. A metaxenial or neophyotic effect on the hybrid embryo, whereby the maternal "chromosome environment" of the mare represents a better substrate for development of the hybrid than the "chromosome environment" of the jenny might be a possible explanation.

The recent work on the distribution of growth hormones in dwarf maize and in "lazy" maize shows how the production, diffusion, and destruction of important growth controlling substances takes place in situations simple enough to be analyzed. Possibly we have very much underestimated the potency of the interactions between embryo and the surrounding maternal tissue. The situation in Citrus, where the embryo-sac seems to be able to wipe clean the slate of differentiation, and to start growth afresh at the embryonic level offers a challenging point of departure for further research.—EDITOR.

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GENETICS CONGRESS TRAVEL PLANS

AN announcement regarding preliminary arrangements for travel to and from the International Genetics Congress at Edinburgh (August 24-30, 1939) has just been issued, by Dr. E. W. Lindstrom, who is serving as American Representative for Congress transportation. Arrangements can be made through Dr. Lindstrom, Iowa State College, Ames, Iowa, who will have the cooperation of the travel service of Edward S. Allen and John H. Harms, 509 Welch Avenue, Ames, Iowa, representatives of all Conference lines.

Passports — United States passport costs $10. Applications must be executed personally in the United States Passport Agency in San Francisco, Chicago, Boston, or New York City at the Passport Agency, Manhattan or the offices of the Clerk of the County Courts in the other boroughs; outside of these cities, applications may be executed before the clerk of any court (district or state) authorized to naturalize aliens. One passport suffices for husband, wife and minor children.

Visas — Great Britain, $2.00; France, $2.09 (transit visa, valid for visit of 15 days, $.34); Germany, $.50.

No fees for Norway, Sweden, Denmark, Belgium, Czechoslovakia, Netherlands or Switzerland. Visas may be secured through travel agents or direct from consuls.

United States Revenue Tax — $5.00 round trip.

Steamship Passage — A few rates, round trip, are the following:

1. ANCHOR LINES: New York-Glasgow-New York.

Cabin	Tourist	Third
$322.50	$244.50	$171.50

2. CANADIAN PACIFIC:
Empress of Australia and *Duchess*, steamers: Montreal-Southampton; Glasgow-Montreal.

Cabin	Tourist	Third
$343.00	$248.50	$175.75

Empress of Britain

Cabin	Tourist	Third
$473.50	$275.00	$184.75

3. CUNARD WHITE STAR:
Britannic and *Samaria:* New York-London; Liverpool-New York.

Cabin	Tourist	Third
$348.00	$250.00	$177.00

(*Continued on page* 448)

NUCELLAR EMBRYONY AND JUVENILE CHARACTERS IN CLONAL VARIETIES OF CITRUS*

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IN most forms of Citrus, the embryos are in part gametic (produced by the ordinary sexual process), and in part nucellar (produced asexually, from cells of the nucellus). Both these methods of seed reproduction bring about conspicuous vegetative invigoration, called *neophyosis* (Greek, "causing to grow anew") by Swingle,[19] which declines with increasing age of the tree or clone from seed.† The thorniness which is characteristic of Citrus seedlings is shown about equally, on the average, by gametic seedlings and nucellar seedlings, and therefore it is inferred that the two methods of reproduction are about equally effective in producing seedling rejuvenation. Their relative rejuvenating effectiveness can not be directly determined from the size and rapidity of growth of the two kinds of seedlings from the same parents, because of the great genetic variability which results from sexual reproduction in polyembryonic forms of Citrus.[8, 25] Gametic seedlings from selfing, or from certain intraspecific crosses between closely related varieties, are usually inferior in vigor to nucellar seedlings of the same parent or parents. On the other hand, hybrids from wider crosses are often about equal and occasionally definitely superior in vigor to the corresponding nucellar seedlings. It is probable, however, that, excepting possible occasional effects of seed-borne infection or heritable somatic variation, any clonal variety originally derived from a gametic seedling is fully restored to its original seedling vigor whenever it is reproduced by nucellar embryony. Certainly, marked nucellar-seedling rejuvenation can be observed within a given clonal variety, such as the Valencia orange or the Eureka lemon, and increased vigor is transmitted in successive propagations by budding over a considerable number of years.

As a result of nucellar embryony, a clonal variety of Citrus may include an indefinite number of nucellar-seedling clones,‡ of various ages from seed. Therefore, trees of a given variety, all of the same age from budding but belonging to clones of different ages from seed, may be grown together. Since the clones thus compared are genetically identical, aside from possible genetic variation in somatic cells, Citrus provides very favorable conditions for investigation of the problem of age changes in clones.

Comparisons of this sort have been made at the Citrus Experiment Station, near Riverside, California, and differences in vigor and fruitfulness between old and young clones of the same variety, similar to those reported by Hodgson and Cameron,[11, 12] have been observed.

Experimental Evidence of Clonal Senescence in Citrus

Trees budded from young nucellar seedlings of the Paper Rind (St. Michael) orange and the Valencia

*Paper No. 391, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

†See the introductory discussion in the accompanying paper by Hodgson and Cameron.[12]

‡The term *clone* is here used to designate the descendants, by artificial vegetative propagation, of any single Citrus seedling, whether this seedling is derived from a gametic embryo or from a nucellar embryo. Clones which are genetically identical then belong to the same clonal variety. Such clones may be called *seedling clones* for distinction from the use of *clone* for propagated lines or strains of bud-selection origin.

orange were set in a trial orchard in 1920 and 1921.* (For the Paper Rind, the stocks were trifoliate orange and sour orange; for the Valencia they were Rough lemon and sour orange). These trees of young nucellar-seedling clones have been incidentally compared with old-clone trees in the same orchard, which had been budded from ordinary orchard trees of these old varieties, as follows: two trees of Paper Rind on Rough lemon set in 1921, and two of Valencia on sweet orange set in 1923. The young-clone trees have been conspicuously more erect in growth, and have appeared much more vigorous and less inclined to early fruiting. In June, 1929, the average number of seeds per fruit, in 20 fruits each of three Paper Rind trees, was as follows: one old-clone tree (on Rough-lemon stock), 18.45; one young-clone tree on sour-orange stock, 6.15; one young-clone tree on trifoliate stock, 8.75. The average of the young-clone numbers, 7.45 seeds per fruit, is 40.4 per cent of the old-clone number. These numbers are somewhat higher than Hodgson and Cameron's[12] for the same old clone, and several times as high as theirs for young clones, giving a much smaller but still very striking percentage difference. This lower seediness under coastal climatic conditions agrees with Coit's[6] observations on the lower seed production of the Washington Navel orange at Whittier, Los Angeles County, than at Riverside, with artificial cross-pollination. A few recent records on several hybrid varieties grown near Tustin, Orange County, and at the Citrus Experiment Station, Riverside County, show a similar tendency; with different varieties, trees, and seasons, the Tustin number has ranged from about one-tenth of the Riverside number to approximate equality with it.

Experimental Results

A few typical results will be presented here from a special experiment on seedling rejuvenescence in old clonal varieties. This trial included three varieties of the mandarin group (including the King mandarin), seven varieties of sweet orange, two varieties of lemon, and three varieties of grapefruit and shaddock. All these varieties were budded in the spring of 1930; the stocks were sweet orange for the lemons, Cunningham citrange for the satsumas, and sour orange for all other varieties. With each variety, six stocks were budded from an old-clone tree and six from a young-clone tree; three stocks were budded from the upper part of the head of each parent tree, and three from the lower part. Each parental young-clone tree had been budded at some time during the period 1917 to 1920 from a nucellar seedling two or three years old. Each parental old-clone tree, except with several varieties included in the experiment but not especially mentioned below, was the seed parent of the nucellar seedling of the same variety which has just been mentioned, and had been planted about twenty years previously as an ordinary budded tree of its variety.

Part of the satsuma trees were transferred to the orchard about two years after budding. With the other varieties, after about three years in ordinary nursery rows, records were made of trunk circumference, fruit yield, and flowering. Table I gives results for several typical varieties. The six young-clone progeny trees of a variety usually averaged larger than the corresponding old-clone progeny trees, and showed much less tendency to blooming and fruiting (Figure 5). They invariably averaged much more thorny than the old-clone trees. The trees budded from the upper part of the parental young-clone trees, with selection for thornless or least-thorny budwood, regularly averaged less thorny than the trees budded, without selection for thornlessness, from the lower part of the same parents. Usually, however, they were more thorny than the old-clone trees. With the old-clone progeny trees, which usually were only slightly or very slightly thorny, the location of the budwood on the parent tree had no noticeable effect on thorniness. There seemed to be a tendency, in both old and young clones, for the trees budded from the upper part of the parent tree to be the more productive of fruit set at two years from budding, and also a considerable

*The species mentioned in this paper are as follows, according to Swingle:[20] grapefruit, *Citrus paradisi* MacF.; lemon, *C. limon* (L.) Burm. (the Rough lemon may be an interspecific hybrid); mandarin, tangerine, and satsuma, *C. reticulata* Blanco (the King appears to be a hybrid of mandarin and sweet orange); sour orange, *C. aurantium* L.; (sweet) orange, *C. sinensis* Osbeck; trifoliate orange, *Poncirus trifoliata* Raf. The citranges are hybrids between the last two species.

With all four varieties discussed on this page, the thorniness of the especially thorny orchard trees is largely limited to an inner zone of branch growth evidently produced in about the first three seasons after orchard setting, as has been described for the Kara. Outside this zone, however, these trees commonly are considerably more thorny than the trees from thornless budwood.

tendency, in the young clones, for these trees to bloom more heavily at three years from budding.

Trees of some of these varieties were set in an orchard planting in 1933 (the satsumas in 1932), and have continued to show the same sort of differences in growth, blooming, and fruiting as in the nursery. In the lemon varieties, the young-clone trees are now (1937) moderately larger; in most other varieties, the difference in top volume is strikingly large. In all varieties, the young clones have bloomed considerably less abundantly than the old clones.

Several thousand seedlings, more than two-thirds nucellar and the rest mostly interspecific hybrids, mainly very thorny, were set in a crowded planting (2×5 feet) when about three to five years old, and grown for about ten or eleven years (until 1930 and 1931), with repeated severe heading back. After each "dehorning" the thorniness of the new growth seemed undiminished, even after the time (May, 1930) when larger orchard trees, which had been budded in the period 1917 to 1920 from some of these seedlings, furnished thornless budwood for the special rejuvenescence experiment described above.

One of these seedlings, which was the original tree of the Kara mandarin,[10] and two nucellar seedlings of this variety from sowing in 1927, were set in the orchard in 1931 and 1932, respectively. At present (December, 1937) all these trees are very thorny, on branches of all sizes, for about one meter from the base of the primary branches, while the highest branches are largely thornless. Two trees budded from the original Kara seedling in 1925 and 1926, and set in the same orchard in 1932 and 1930, are similarly thorny.

Two trees were budded from the original Kara seedling in 1918, when it was about three years old, and were set in the orchard in 1920. By the fall of 1925 these budded trees were similar, in amount and distribution of thorniness, to the present condition of the Kara seedlings just described. Budwood was selected for thornlessness, in the largely thornless upper part of these two trees, in four years from the fall of 1925 to the spring of 1930, and the resulting trees regularly had little thorniness in nursery and orchard.

Budding from various other F_1 hybrid seedlings of similar age, in the fall of 1924 and of 1925, regularly produced very thorny nursery trees. In 1925 (fall) and 1926 (spring), certain hybrid varieties were budded both (a) from the seedlings and also (b), on adjacent stocks, from budwood selected for thornlessness or low thorniness on orchard trees which had been budded from the respective seedlings about six or seven years previously. The average thorniness resulting was much lower with the trees from the selected orchard budwood, although in some varieties little or no difference was observable. In 1928 to 1930 various promising hybrids, partly the same as were budded in 1925-26, were propagated from orchard budwood selected for thornlessness, with better average success than before in obtaining budwood from completely thornless shoots or branches. In most cases, nursery trees with comparatively little thorniness were produced.

In 1928, with four hybrid varieties, in addition to the usual propagation from thornless budwood taken from high branches of orchard trees, two or three trees of each variety were budded from very thorny budsticks, apparently of similar age, selected from the generally more thorny lower part of the same trees. With all four varieties, the thorny budwood produced much more thorny nursery trees (Figure 7). Two trees of each variety from thorny budwood were set in the orchard, and these now (December, 1937) are all much more thorny than the adjoining trees budded at the same time from thornless budwood. With two varieties, trees budded from the original seedling in 1925-26 are available for comparison. With the Kara mandarin, these trees (the same as have been compared with seedlings above) are very much more thorny than those from the thorny budwood of 1928; with the Wilking mandarin,[10] one tree budded from the seedling is much more thorny than the trees from thorny orchard budwood, while the other about equals them. Finally, two Wilking trees, budded in 1925 from orchard budwood selected for the nearest approach to thornlessness, now average decidedly more thorny than the trees budded from thornless budwood in 1928, but are considerably less thorny than those budded from thorny budwood in the latter year.

With one of the four varieties (the Ging tangemon) in the test just described, the thorny and thornless budsticks were taken from the same unbranched shoot. This was an erect "watersprout," about 2 meters long, which arose on a pruned branch about ½ meter from the ground and probably was entirely produced in 1927. Most of the lower half was extremely thorny, while the upper half had only occasional traces of thorns; the two budsticks were more than a meter apart. The differences in thorniness of the progeny trees have been striking (Figure 6).

Thorniness an Index of Tendency to Vegetative Growth

Altogether, the evidence is very convincing that the tendency to thorniness declines gradually in clones derived from gametic seedlings, and that different grades of thorniness may be transmitted by budding at the same time in the same clone. The same conclusion is strongly indicated for nucellar-seedling clones also by the special experiment of 1930 which has been described.

The evidence here presented indicates that, in the propagation of promising new varieties, the reduction of thorniness can often be greatly promoted by selection of thornless budwood on trees which have grown about five to ten years in the orchard.

Citrus thorns are abortive branches, which sometimes bear leaves and even flowers,[16] and vigorous shoots are in general more likely to be thorny than are weak shoots. These facts create a presumption that seedling thorniness is a manifestation of a special tendency to vegetative growth, as seems obvious with all the other general juvenile characteristics of seedlings which have been observed — although even weak shoots of young seedlings are commonly much more thorny than strong shoots of old-clone trees. This point is especially important, since the evidence of early and gradual clonal change is most complete for thorniness. The comparative growth rate at different times is much more difficult to determine adequately, and the main point so far established is the conspicuously greater vigor of young nucellar seedlings and nucellar-seedling clones, as compared with clones of the same horticultural varieties whose age is usually not at all precisely known but which is surely very much greater.

Our experiments indicate that seedling vigor is more persistent than seedling thorniness, and that the rate of decline of thorniness has no uniform general relation to the initial amount of thorniness. There are great differences among genetic types, both in the initial thorniness of seedlings and clones derived from young seedlings, and in the rate of later decline of thorniness. Genetic types which seem to produce no thorns at all have been found, but seem to be exceptional. In nucellar-seedling clones of Satsuma, the thorniness of the new growth on orchard trees budded from two-year-old nucellar seedlings in 1918 was usually slight at first and soon disappeared practically completely. Nevertheless, the trees that were budded in 1930 from two such orchard trees for the special rejuvenescence experiment still show conspicuously superior vigor. In nucellar-seedling clones of sweet-

REJUVENATION BY NUCELLAR EMBRYONY IN AN OLD ORANGE VARIETY

Figure 5

Paper Rind orange trees; June 15, 1933, about 37 months after budding. *A*—Budded from an ordinary (old-clone) orchard tree; left three trees from low budwood, right three trees from high budwood. *B*—Budded from a young-clone orchard tree which had been budded from a two-year-old nucellar seedling in May, 1917; budwood taken as for old clone. Note the greater vigor of the nucellar clone.

orange varieties, of about the same age from seed, the superior vigor is now similarly marked, but the seedling thorniness, initially high, still persists to a large extent. For example, the Trovita orange, which resembles nucellar-seedling Washington Navel in thorniness and vigor, has a large average amount of thorniness on trees which were budded from orchard budwood selected for thornlessness in 1928. On some of these trees, thorns are abundant even on a large part of the recent growth.* In striking contrast to the Trovita, the original (gametic-seedling) clone of the Kara mandarin, which for some years resembled the young orange clones in thorniness, produced practically thornless trees from selected orchard budwood in 1925-26, at 10½ to 11 years from the germination of the seed.

In the cases where trees of one young clone have shown different amounts of thorniness, according to the source of budwood, corresponding differences in rapidity of growth have not usually been distinguished. This may be merely a result of the slower decline of the growth rate, together with greater difficulty of adequate determination. There is, however, considerable evidence that the tendency to bloom and set fruit increases as the thorniness decreases, and this is probably an illustration of the well-known principle that the tendencies to reproductive and to vegetative activity are inversely affected by certain changes in metabolic balance.

The fruits of seedlings and young clones of satsuma (supposedly Owari) seem earlier in ripening and better in quality, according to comparisons which so far have mainly involved trees of different ages and in different fields. Table II shows differences in fruit-juice composition between young-clone trees, giving their first full crop, and old-clone trees set several years earlier in the same field. The young-clone fruits tended to be more clustered on the trees, and, perhaps largely on that account, they tended to be smaller, thinner-rined, more juicy, better colored, and sweeter.

The tendency to alternate bearing seems much more marked in young than in old clones, evidently because the presence of a crop of fruit is much more likely to prevent the formation of flower buds and setting of fruit in young clones. This tendency has been marked with

Comparisons in Ruvel and Washington Oranges

The Ruvel orange is a navel variety of unknown origin, very different from the Washington Navel, of which an old tree was found at the first site of the Citrus Experiment Station in Riverside. Two old-clone trees and four young-clone trees of the Ruvel were set in a Station orchard in 1930. The young-clone trees have all usually produced considerable crops of fruit in the last four seasons; while the old-clone trees, which are decidedly smaller, have bloomed much more heavily but so far have produced only a very few fruits in any season. In the Coachella Valley, under hot desert conditions, two old-clone trees have been less vigorous and productive than adjacent young-clone trees, although the former have been more productive than at Riverside. In Orange County, under coastal climatic conditions, the old-clone trees average nearly as large as the young-clone trees, and have been decidedly more productive of both flowers and fruit than the young-clone trees. Evidently the Ruvel agrees with the other varieties tested in having more vigorous growth and much less abundant flowering in young clones, although, according to the cultural conditions, early fruit production may be either higher or lower in the young clones.

With both the Ruvel and the Washington, the average size of the "navel" structure, composed of a supernumerary whorl of carpels, has been found to be considerably smaller in young clones than in old clones.[9] The fruits of young clones seem to have a fairly general tendency to greater elongation, greater firmness and ragginess of pulp, and earlier onset of over-ripeness changes, including core hollowness, rind puffing, and pulp granulation and drying. These tendencies, together with the long persistence of excessive thorniness which seems to be characteristic of oranges, probably make young clones horticulturally inferior within the Washington and the Valencia, although young clones seem superior in the Ruvel under some climatic conditions.

*Only part of the Trovita trees budded in 1928, 1929, or 1930 have yet been examined in the orchard for thorniness. One tree budded in 1930 has little thorniness.

THORNY AND THORNLESS TREES FROM AN UNBRANCHED SHOOT

Figure 6

Trees of the Ging tangemon, all budded in 1928 from one unbranched erect shoot on an orchard tree. *A*—Two trees from budwood taken near middle of upper half of shoot, this half being almost completely thornless. *B*—Two trees from budwood taken near base of shoot and bearing thorns about 3 to 5 cm. long.

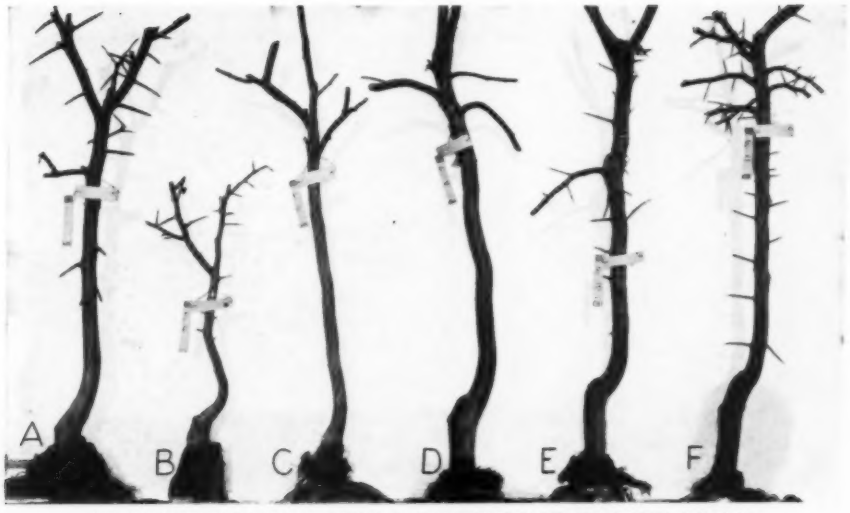
young trees of young clones of satsuma; if it does not persist too long, the young clones may prove decidedly superior, because of such characteristics.

Clonal Senescence Seems General and Non-Genetic

Traub and Robinson[22] say (p. 795), "There are no facts to prove that citrus clones are subject to senescence," and suggest that any demonstrated invigoration shown by nucellar seedlings may be due to genetic change of some kind. The evidence now available, however, from the observations of Webber,[24] Swingle,[18,19] Tanaka,[21] Hodgson and Cameron,[11,12] and the present writer, together with the general knowledge of the thorniness and slowness to begin fruit production which characterize Citrus seedlings, seems to make the conclusion almost unavoidable that certain age changes in Citrus clones *regularly* occur, and that they are *regularly* and *completely* erased by asexual seed reproduction. If this is true, these changes can hardly be genetic in the usual sense of the word, but they nevertheless do present a problem of great interest for plant breeding. Any prospect of gradual and considerable age changes in such characteristics as growth vigor, fruitfulness, and shape of fruit, in new clonal varieties, should interest plant breeders, as should also the possibility, with Citrus and perhaps with certain other plants, of abolition of those changes through asexual seed reproduction. The possible relation of such changes to senescence in animals is also of especial interest to general physiology.

The Probable Nature and Causation of Clonal Senescence

The available evidence seems suffi-



THORNY BUDWOOD TENDS TO PRODUCE THORNY TREES

Figure 7

Kara mandarin trees, prepared for orchard setting: August 13, 1930. Sources of budwood and times of budding as follows: *A*—gametic seedling, 1925 (fall); *B*—same as *A*, 1926 (spring); *C*—orchard tree budded in 1918, thornless budwood, 1925 (fall); *D*—same as *C*, except budded (this tree) in 1928 (spring); *E-F*—same as *D*, except from thorny budwood.

cient to justify discussion of some of the more obvious considerations relating to the possible causation of these age changes.

Artificial cultures of embryonic cells of higher animals, in which the cells do not become differentiated into tissues and organs, evidently undergo no senescence if the cultural conditions are kept sufficiently favorable. This seems to demonstrate that the senescence which inevitably occurs in every animal body is entirely a result of its differentiation, which ultimately always produces an unfavorable environment for its cells.[3, 4, 26]

Minot[13] especially developed the hypothesis that senescence is primarily caused by the normal processes of differentiation, which go too far and so become injurious. Robertson[15] assumes the occurrence of competition between cells and tissues of different grades of differentiation, to the disadvantage of the less differentiated cells and ultimately of the whole body. Any changes in such features as nucleus-cytoplasm ratio, relative amount of non-living structural materials, and concentration of hormones or catalysts, are evidently incidental to cell and tissue differentiation. Other possible agents of age deterioration in animals are essentially accidental; they include inadequate nutrition; disease viruses, other foreign proteins, and toxic substances in general; and inert waste products. Differentiation, with the necessary exposure of the organism to the external environment, obviously tends to various injuries of this accidental sort.

As Molisch[14] shows, such considerations apply in part, at least, to individual plants. Benedict[2] emphasizes, however, that a plant clone is carried on by essentially undifferentiated meristematic cells, so that any age changes in a *clone*, as distinguished from age changes in individual plants, must be persistent and self-propagating even in rapidly dividing cells. Obviously, some of the old-age limitations and dangers of the individual plant do not affect the clone as such; on the other hand, presumably any age

change, except possible specific stock effects, which may affect a clone may occur also in an individual seedling plant if this attains sufficient age and size.

Bailey[1] concluded, more than 40 years ago, that true old-age deterioration, or "wearing out," of clonal varieties, had not been demonstrated. Benedict,[2] however, has since found apparently convincing evidence that certain definite age changes, which he interprets as involving some reduction of biological efficiency, occur in seedlings and in clones derived from seedlings, in various genera of woody plants.

Infectious diseases caused by filterable viruses are known to be a common cause of accidental clonal deterioration — for example, in potatoes. Can such diseases, regularly transmitted by budding but not by seed, furnish the sole explanation of clonal weakening and nucellar-seedling reinvigoration in Citrus? There is evidence that virus diseases affect Citrus. Fawcett[7] has secured evidence indicating that Citrus psorosis (California scaly-bark) is a virus disease, which often becomes systemic in the tree, and then is transmitted by budding and produces both bark and leaf symptoms. Fawcett[7] and Smith[17] also mention several other Citrus diseases suspected of virus causation. At Riverside, certain lemon hybrids, and perhaps very rarely other hybrids of recent origin, regularly show a mosaic disease which does not seem to be otherwise known on Citrus.

Several facts, however, combine to render the hypothesis of virus infection improbable as a complete explanation of clonal senescence. First, while a thorough search has not yet been made, symptoms of virus disease have not been incidentally observed on the old-clone trees concerned in the special experiment described above. Second, the onset of the age changes seems to be universal and gradual. Third, the decline of thorniness is much more rapid in some clonal varieties than in others grown with them. Fourth, thorny seedling trees, and trees budded from young thorny seedlings, retain the potentiality of producing very thorny shoots in their trunks and the lower part of their main branches for many years after it has disappeared from the outermost zone of branches. If viruses are the sole cause of the apparent senescence, their effects must increase gradually outward, leaving the trunk, for many years, much less if at all affected.

It seems fairly safe to eliminate from serious consideration the simple accumulation of inert materials in the cells. The evidence not only indicates that the juvenile and senescent conditions are alike propagable by the bits of tissue employed in budding, but it also indicates that the old-age condition is actually promoted by length growth of shoots. This senescence (if such it should be called) therefore seems to be favored by repeated cell division, rather than by aging of individual cells.

This fact suggests that distance from the roots, erect position of shoots, and full exposure to external agencies, such as sunshine and wind, are possible factors of age change. The changed condition appears to be fully or largely transmissible in cell division, and therefore it evidently concerns substances or states which are reproduced in cell division.

Many kinds of woody plants show various kinds of changes from juvenile to mature or from vegetative to fruiting types of growth, and in some cases at least these definite stages of differentiation can be propagated vegetatively.[5] One speculation may be worthy of mention in this connection. Certain changes in important cell proteins, resulting from conditions of the "internal environment" of the plant, may be somewhat persistent and to some extent self-propagable, although not infectious. This hypothetical self-propagation may seem more plausible if we locate the changes in the plastids, although that assumption seems unnecessary. Age or growth changes on such a basis would have two obvious general differences from certain infectious diseases due to crystallizable foreign proteins: (1) the agent would be autogenous, not of foreign origin; (2) it would be transmitted only in cell division. There would be a

resemblance to viruses which are not seed-borne, however, in that the agent would be in some way eliminated or destroyed either in embryo formation or in the mature seed.

Swingle[19, 19a] suggests that neophyosis is caused in some way by environmental conditions surrounding the embryos in the embryo sac (perhaps hormones of the sac apparatus), which affect gametic and nucellar embryos alike, and which seem to be responsible for the fact that embryos are formed at all. This conception implies an active influence which is especially potent in the embryo. Professor Hodgson has called the author's attention to the implication of recent work on plant hormones, which suggests the possibility that rejuvenescence may be associated with a high hormone content of young clones and that senescence may be an expression of its eventual exhaustion brought about by cell division. It has been discovered that dwarf forms of maize are due to a low auxin content.[23] A similar deficiency might prevail in senescent tissue. Child[4] (p. 436) mentions "Bütschli's suggestion that death is due to the exhaustion of the supply of a certain substance—the 'life ferment' —which is gradually used up during life." If a special hormone is related in some such way to clonal senescence and seedling rejuvenescence, it evidently must be multiplied in cell division, possibly through an autocatalytic action of the substance itself. We need only assume that the conditions are somewhat less favorable for the production of such a substance (or substances) or more favorable for its destruction, in tree growth than in embryo formation, so that a gradual decline takes place, through a period of years, as shoot growth advances. Stanley's[17a] discussion of the reproduction of virus proteins suggests possibilities of this sort.

Possibly no single kind of agency is entirely responsible for the changes under discussion. The problem now seems definite enough, however, and the knowledge of viruses, hormones, and plant proteins sufficiently advanced, to encourage chemical and physiological investigation. Comparative biochemical studies of old and young clones seem desirable.

Summary

It seems to be well established that:

1. Both gametic and nucellar seedlings of most genetic types of Citrus are at first very thorny. This tendency to thorniness gradually declines with continued extension of growth in seedling trees and in clones propagated from seedlings, but it declines much less rapidly, if at all, in the trunks of the seedlings.

2. Different grades of thorniness in the same young nucellar-seedling clone tend to be propagable by budding.

3. Reproduction of an old seedling clone by nucellar embryony regularly produces a more vegetative condition, with more vigorous growth and less tendency to flowering. This rejuvenated condition lasts for many years, usually long outstanding seedling thorniness.

4. As thorniness declines, the tendency to flower production and usually to fruit setting increases. These and other visible changes which characteristically occur with increase of clonal age, are of the same general nature as those which occur from youth to maturity in individual budded trees.

5. The changes with age and the reverse changes in seed reproduction are not genetic.

It is probable that:

6. Seedling thorniness, like rapid growth, is a manifestation of a more vegetative condition, which is produced to about the same average extent in gametic and nucellar seedlings. The decline of seedling thorniness is associated with repeated cell division, rather than with age *per se*.

7. The regularly occurring age changes are not due to recognizable infectious diseases or to simple accumulations of inert materials in the cells. Fundamentally these changes are temporary but persistent modifications of the meristematic cells, which are propagated in ordinary somatic divisions but erased in seed formation. The mechan-

isms which might bring about such changes are discussed.

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Table I. Characteristics of three-year-old budded trees of old and young clones of three citrus varieties (12 sets of 3 trees each)

Variety	Clone and budwood	Mean trunk circumference (cm)	Total fruits*	Bloom †
Eureka lemon	Old (high)	155	26	Heavy, medium, very light
	Old (low)	149	26	All medium
	Young (high)	135	1	All extremely light
	Young (low)	144	0	Very light, extremely light, none
King mandarin	Old (high)	81	44	Heavy, medium, extremely light
	Old (low)	87	44	Two very heavy, one extremely light
	Young (high)	112	18	All heavy
	Young (low)	103	0	All very light
Paper Rind orange	Old (high)	188	119	Two very heavy, one heavy
	Old (low)	99	57	Two very heavy, one light
	Young (high)	110	0	Very heavy, heavy, medium
	Young (low)	121	0	Medium, light, very light

* Total yield of set of 3 trees in each case.

† Abundance of flowers, by individual trees.

Table II. Size and composition of fruits of seven old-clone and young-clone satsuma trees, picked November 26, 1935 (Random samples of 20 fruits each)

Tree		Fruit			
Top*	Stock	Average weight (grams)	Juice content (%)	Soluble solids in juice (%)	Citric acid in juice (%)
Old clone	Trifoliate	109.8	41.4	10.2	1.12
Old clone	Trifoliate	104.6	42.0	10.2	1.01
Old clone	Savage citrange	87.6	43.7	10.7	1.02
Old clone	Savage citrange	89.2	43.7	10.9	1.01
Seedling	(None)	85.8	46.0	12.0	0.98
Seedling	(None)	74.6	45.4	11.1	0.85
Budded from a seedling	Trifoliate	79.0	48.7	12.3	1.01

* All supposed to be Owari. The seeds which produced the seedlings all came from one variety-collection tree; the budwood which produced the last two (perhaps all) of the old-clone trees came from other sources, but it was all taken from ordinary orchard trees.

EUGENICS AND FARM LIFE

A Review of Three Recent Publications

HERE are three "musts" for the reading-tables of those, especially youth, who are considering whether to undertake a country life or to persevere in one already embraced.* The three evaluations of farming are completely contrasting among themselves.

From Taylor and Taeuber of the U. S. Department of Agriculture comes more truth than we have right to expect from anybody except the Lord of Truth Himself. Their maps from the Bureau of Agricultural Economics show which U. S. farming counties are disadvantaged temporarily or perhaps permanently,—on seven different criteria, and there is an eighth map to help you avoid counties that fall down on three or more criteria. A final short passage most lucidly summarizes the various "farm programs," tried and to be tried.

Even so, the authors leave me in doubt about their main point: that 'live-at-home' farming on the best lands as well as on the relatively poor is necessary because "complete economic specialization and geographic division of labor in agricultural production increases freight, storage, financing and middleman costs to such an extent that farmers of one region do not provide good markets for family consumption goods grown in other regions." Does this argue that every farm family should produce *both* its eggs and butter and quilts and tables, or that every farming *county* should do so?

I vote for the latter only—after thirty summers spent upon the farm seeking to raise a family on "plain living and high thinking," that is, on a city standard stripped of all vanities and non-essentials. Mere physical well-being as city people understand it leaves no time for both eggs and butter, etc., etc., plus even a small cash crop. My neighbors who thus diversify are always ailing, are too sleepy to read for an hour in the evening, and their daughters by fourteen years of age are misshapen with toil. Moreover, cooperation and intercourse among themselves is the great spiritual need of country life and this should be built up through hearty patronage of the neighbors' specialties. At present, interfamily feuds leading one to say, "I wouldn't buy eggs of *her* if I never ate eggs again," are the bane of country life, second only to the mordant caste-feelings of many a county seat against its constituency and, of course, of each race against others. When bankers and store-keepers, Grange leaders and farm wives shall unite to "Buy-at-Home" and organize for full profitable employment of their own youth through home-consumption and service-trades within their own area, country life may take on a civilized humane business-dignity.

You may object that the labor of farm wife and children is, and must be, most economically employed right where it is, that is, within the home, producing a fine diet, and clothes, etc., even if a little queer in cut. To this we reply that where any skilled home-crafts have not already been lost it is well to preserve them, in either city or country. But where they are already departed, specialized production within the farm neighborhood and Buy-at-Home is the thing to urge. If fine people are to be kept from migrating from the country to the city, we must

*1. TAYLOR, CARL C., and CONRAD TAEUBER. Constructive Rural Farm Population Policies, The Millbank Memorial Fund Quarterly, Vol. XVI, No. 3, July, 1938, 40 Wall Street, New York City. 20 pp.

2. SMART, CHAS. ALLEN, *"R. F. D.,"* Book-of-the-Month, Norton & Co., 1938. 314 pp.

3. FRITTS, FRANK and RALPH W. GWINN, "Fifth Avenue to Farm," Harper, 1938. 282 pp.

get rid of the present double standard for farm-*vs* city-wives. Drs. Taylor and Taeuber should make it plain that no more and no less should be expected of a farm-wife than of a city-wife. For instance, when times are very hard and both wives have a little unemployed time and both need a couple of new bed-coverings, shall both of them be expected to foreswear baker's bread, buy flour in sacks (to get the sacks and to save by home baking the cash to get the thread, etc.) and then spend many days, the one raising ducklings for their down and the other searching the wholesale district for a cheap filling, to enclose within the sacking to make quilts? If one, then both, say I.

Or you may agree that Buy-at-Home (*not* Live-at-Home) is the right text for the sermon, and nevertheless you may object that all such idealistic homilies are really in vain since nothing on earth can make countrymen get together. It is indeed many, many years since a voice from another bureau of the Department of Agriculture, that of Plant Industry, was lifted in the wilderness of governmental bureaucracies and began to urge community organization for overpowering reasons neither spiritual nor marketwise. It was shown that solid botanical and genetic realities demand what the "rural sermonizers" are wont to preach. At least in cotton farming, almost our most peonizing industry, organization in units no smaller than the community is essential.* Few individual farmers operate on the grand scale today, to the extent that they can afford to gin their own cotton. The shift to public gins put in operation a devilishly efficient device for mixing the seed stock of all the farmers in a community. Thus the cotton breeders' efforts to improve the crop were set at naught, and in a community where even two or three varieties were grown, let alone a dozen or a score, mixing of seed at the gin made it impossible for any farmer to maintain a uniform seed supply. With public gins and 100% individualism the American Cotton Belt could never hope to add *quality* to the huge *quantities* of inferior cotton produced—which the government seems now to be "buying" with 8-cent-a-pound loans.

Where only one variety was raised in a community, this problem of mixing of varieties was automatically solved,—and with resulting economic advantages which were almost as compelling. No individual farmer, raising only one or a few bales of cotton could hope to bargain effectively even if his crop were far above average. An entire community, producing a uniform, superior product could gain much by collective bargaining in selling as a unit. The same organization which acted as a bargaining agency could easily be used to further community activities in other ways,—it could be the nucleus for the buy-at-home movement whereby secondary crops might be distributed among the neighbors.

Somehow these same principles would seem to apply, with varied modifications, to many farm products,† and might give the impetus so much needed to hasten community organization, which it seems we must have if farming in America is going to remain a *good life* under twentieth century conditions. The fact that by 1935 three hundred communities in the Southern states had organized along these lines shows that the people's minds can move ahead as fast or faster than some of the Bureaus have moved—for the one-community crop plan has never been formally adopted as a policy by the Department of Agriculture as a whole.

But it is all not fast enough. If Fritts and Gwinn are right that farm life is the only antidote we now know for race suicide, while Smart is right in blessing the

*Cook, O. F., Unified Cotton Communities—The study of Heredity Leads to Reorganization of an Industry. *Journal of Heredity*, 16:1678, 1924.
The One Variety Community Plan. U. S. Department of Agriculture Yearbook for 1936, pages 696-697. Washington, 1936.

†The advantages of livestock breeding on a community basis are discussed by J. L. Lush in his recent book, *Animal Breeding Plans* (Collegiate Press, Ames, Ia., 1937).

farm for a hundred things and damning it as a way to make a living, then somebody really should get busy. An inventive attack on ways and means to make rural organization come to life in perhaps the finest challenge open to patriots at the present moment. For it would likely revolutionize breeding among our plants and animals *and* among our humans as well as revive the rural "body economic" which seems just now quite as "run out" and in process of being mongrelized (with government) as was ever any species of living creature. There seem to be basic evils which must be dug out by the roots. If this is so, is it enough to "sell farm life" to the youth of rural America via the 4-H Clubs and the Future Farmers movement? There seem to be basic inconsistencies in touting farm life as "good," when Taylor and Taeuber can seem to prove, that as things are today, the chances are all against its being so by any adequate criterion in a majority of cases.

In the light of all the above we may wonder whether any other eugenic expedient offers so much of promise as that of maintaining the finest human types on American farms, in a social and economic environment which would make it possible to enjoy the *good life* which these three authors picture so glowingly in their different ways? Such an approach would put teeth into the argument of race conservation which Fritts and Gwinn so eloquently advance—an argument which will never get anywhere merely on the plane of verbal hortation. Jeremiads are all right in their way, but we must have the facts and the will and the skill to invent and put wheels on sociological go-buggies which are well enough built to take the human race places at least as efficiently as Henry's immortal Model T used to take families on week-end tours. Preachments by theorists living in our greatest metropolis, no matter how earnest they be, or enjoying "a splendid living on the farm" by writing a best seller, are very well in their way, but our fine words must be reduced to *action* before our buggies begin to roll very fast. Telling the other fellow what a fine farmer he would make does not get us very far.

The curious and disquieting "nigger in this wood pile" seems to be that since 1911 one branch of the Department of Agriculture has on more than one occasion repeated this obvious formula regarding the need for community organization in cotton, in its compelling simplicity and logic, and things haven't yet begun to happen very fast. Biologically, sociologically, and economically, community organization in our rural areas is what we must have. But here are several fine authors who can write 616 pages and scarcely mention it. This is not perhaps encouraging to those who are struggling hard to stop the erosion of our soils, the breeding of runty animals and plants, and the flight of our most enterprising people to the cities there to die out. The countryside needs to learn dignified human cooperation, which is the hall-mark of modern living and also a main attraction drawing fine young people to our cities even in the very face of death—racial death.

Country-life already has craft dignity and the nobility of fine family life, as our other authors strongly show. But the Book-of-the-Month called *"R. F. D."* is superior to Fritts and Gwinn on the economics of country living — and the latter superior to all contemporary writers on their main point; namely, that it is for the sake of children and the race that one should go from *"Fifth Avenue to Farm."* Mr. Smart, living on his *"R. F. D.,"* apparently involuntarily childless, did not, like the other authors, "inherit a portfolio" of bonds and stocks. So he has learned his economics in a stiff school though with a glad heart loving country-life: "Perhaps I have made it clear by this time that a small farm is a very poor place to earn a living in the ordinary meaning of that phrase and a very rich and splendid place to earn a living in every other meaning of it." Mr. Smart knows that a person working anywhere without a specialty but at the first job brought by chance or whim,

is likely to make merely sweatshop wages—or "minimum wages"—or at best standard wages—all almost equally abhorrent to the college graduate whom the other book is (treacherously?) attempting to entice into the country. To make more than minimum wages anywhere takes a bit of luck or special training or management. This gets demonstrated almost every time that somebody 'has a bright idea'—as for instance 15 ladies who decide to work like mad for 3 hours to make 2,000 sandwiches for a charity ball instead of buying them. They scarcely speak as they hustle and at the end feel very pleased and very productive. But a few minutes with a pencil can usually show that they have 'made' about 18c an hour—sweated wages. That's all right, because they wanted to get together over the sandwiches, anyway, without talking, just as people enjoy a quiet rubber of bridge—with chatter "out."

When Fritz (or Gwinn) and his family leave *Fifth Avenue to Farm* and get together over the string beans to grow and can them—that's all right too, because they too wanted to do it. But their economic reasoning which tried to show that "it paid"—anything more than sweatshop wages—to monkey with those beans, is fallacious. It takes a week of work, and maybe more, to grow and can 100 quarts, which saves you, according to *Fifth Avenue to Farm,* the $12 which such canned goods would have cost in a store. Just about a minimum wage, is $12 for a week's work, and not agreeable to college graduates.

If Fritts and Gwinn had used the words "country living" rather than "farming" in enticing youth to leave Fifth Avenue, and Main Street, theirs would be a sermon to be handed to every youth leaving an American high school. For with eloquence they show that the city with its horrible birth rate will be the death of you in a racial way, while the country will be life to you not only racially but personally if you are a man and not a machine cog or robot. Mr. Smart with all the skill of a professional writer as he is, tells you the same about the personal and craft rewards of life on the *R. F. D.* But Fritts and Gwinn have got the racial meat of the matter in them more than any of the rest of us—more even than Henry Ford (with only his one child and few grandchildren). Henry was of course first to catch the public ear with his grand new doctrine that a countryman with a cash-specialty, preferably industrial, gets subsistence-and-security along with his cash. Add also children and we have indeed the abundant life. Subsistence, security and children are what a farm ought to guarantee you and are what a certain strong faction at the Department of Agriculture is bending its energies to guide you to, with maybe, God willing, a spot of cash too. C. H. R.

BENT-NOSE IN THE NORWAY RAT*

A Study of Interaction of Genes and Diet in the Development of the Character

WALTER E. HESTON

THE bent-nose mutation described below was discovered in the rodent colony of the Zoology Department at Michigan State College in 1930. This name was chosen because it described the character simply and adequately. (See Figure 8.) The rostrum of the nose was bent either to the right or left, and with the exception of abnormalities common to rachitic animals (such as rachitic rosary, pigeon breast, etc.) no other bone anomalies were of frequent occurrence in bent-nosed animals.

Preliminary investigations showed that the character did not manifest itself until some weeks after birth. The youngest animal to show it was 34 days of age, and no animals have been known to develop the anomaly after they were 120 days old. The character usually appeared between the ages of 60 and 70 days. The degree of flexure, measured by the angular deviation of the nose from the sagittal plane of the skull, ranged from 4° to as much as 35° in a group of 66 bent-nosed skulls, with 17.5° as the mean grade of flexure. The frequency of left bends was not significantly different from that of right bends, and there was no statistically significant difference between the mean angles to the left and right.

The literature contains no description of such a character in rats. However, Green and Fekete[6] described two mice which showed a condition identical in external appearances to the bent-nose of the rat. The character might also be compared to "twisted beak" in fowls, reported by Hutt.[12] This is a sub-lethal character which appears to be inherited as a simple recessive.

Bent-noses, when first discovered, were confined to a red-eyed yellow strain of rats derived from Dr. W. E. Castle's stock. It was quite prevalent in this strain. Thus, the facts clearly indicated at the outset that the trait was hereditary.

During 1932 and 1933, a genetic analysis of the character was undertaken which revealed as the cardinal discovery that diet influenced the manifestation of the trait. Matings of the following types were made: bent × bent, bent × normal, $F_1 \times F_1$, and $F_1 \times$ bent. The stock was fed the colony rat ration consisting in general of a cooked diet of various grains, with fruit and meat scraps sometimes added. Occasionally they were given green lettuce, cabbage or alfalfa. Two hundred and four animals were reared, of which 93, or 45.6 per cent, were bent. Then on January 1, 1933, all the rats in the colony were put on the Purina Fox Chow ration as an economy measure. One hundred and ninety-one animals from the same types of matings were reared on this diet, and *not one had a bent nose.* An inherited trait had disappeared! This was ample proof that food was a factor in the development of this character.

Knowing that food was a factor, it was desirable to discover what nutritional agents favored the appearance of bent-nose, and to develop a ration that

*This material was submitted in a thesis to the faculty of Michigan State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author wishes to express his gratitude to Dr. H. R. Hunt of the Zoological Department for helpful guidance in the genetic experiments, and to Dr. C. A. Hoppert of the Chemistry Department who effectively supervised the nutritional work.

could be used in studying the genes involved. If genes for bent-nose could produce their effects only with a certain type of diet, then that diet would have to be used throughout the genetic experiments. An appropriate research procedure was accordingly formulated.

Nutritional Factors Causing Bent-nose

To facilitate the production of a bone abnormality, one would normally experiment with the two main bone-forming elements of the diet, namely, calcium and phosphorus. Some of the early works of Steenbock[1,9,28,29,30], Sherman[22,23,24], and their colleagues have shown that an imbalanced calcium-phosphorus ratio, in the absence of vitamin *D*, gives rise to rickets, a condition accompanied by various bone defects. Such a rickets-producing ration could be made according to several different formulae. The amount of calcium could be high with the phosphorus low—the type of ration usually employed; the amount of phosphorus could be high in the relation to the calcium; or both could be present in too small amounts. Absence of vitamin *D* is essential in any case, since its presence tends to correct the imbalance in the calcium-phosphorus ratio, or in the case of a deficiency of both of the elements in question, vitamin *D* permits a more efficient utilization of these elements.

Calcification may be affected by strontium (Sabel, Cohen, and Kramer[19,20]), magnesium (Orent, Kruse, and McCollum[15]), fluorides (Smith[21,25,26,27] et al), and the flowering sweet pea (Geiger, Steenbock, and Parsons[4]). However, in the study of bent-nose, more stress was laid on the calcium, phosphorus, and vitamin *D* constituents of the diet than on the various other factors influencing calcification.

Rations Tested

Seven rations were used in the experiments on the food factors involved. These included one well-balanced stock ration as a control diet, and six deficient rations. The ration which would produce the highest incidence of bending among the progeny of bent × bent matings, and still not be so inadequate that the animals died before they were 120 days of age, was to be the ration later used in the breeding experiments. The formulae of the seven rations used are listed below.

RATION I—Stock ration—

Corn meal	—30 parts (28.57%)
Oat meal	—30 parts (28.57%)
Milk powder	—30 parts (28.57%)
Oil meal	— 6 parts (5.71%)
Alfalfa	— 5 parts (4.76%)
Yeast	— 3 parts (2.86%)
NaCl	— 1 part (0.95%)

RATION II—High calcium ration—

Corn meal	—38%
Oat meal	—38%
Wheat gluten	—20%
$CaCO_3$	— 3%
NaCl	— 1%

RATION III—High calcium ration plus cod liver oil—

This ration was the same as Ration II with 1 gram of cod liver oil added to each 100 grams of Ration II.

RATION IV—Yeast, high calcium ration—

Corn meal	—38%
Oat meal	—38%
Wheat gluten	—20%
$CaCO_3$	— 2%
Yeast	— 1%
NaCl	— 1%

RATION V—Irradiated yeast, high calcium ration—

Corn meal	—38%
Oat meal	—38%
Wheat gluten	—20%
$CaCO_3$	— 2%
Irradiated yeast	— 1%
NaCl	— 1%

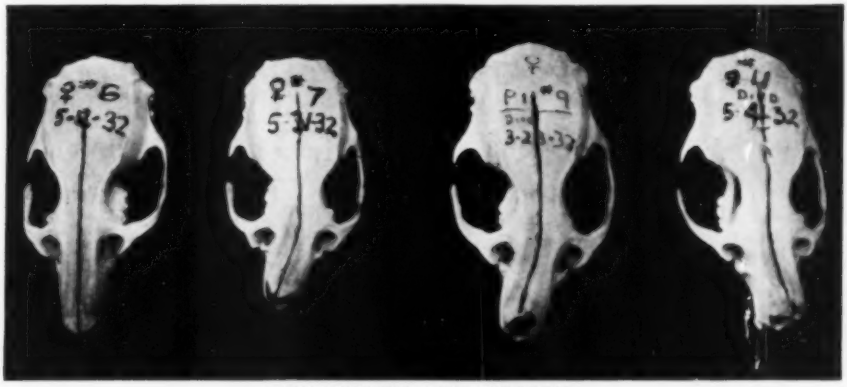
RATION VI—Low calcium ration—

Corn meal	—32%
Oat meal	—32%
Wheat gluten	—10%
Oil meal	—10%
Casein	— 5%
Yeast	— 5%
Milk powder	— 5%
NaCl	— 1%

RATION VII—Low calcium ration—

Corn meal	—43%
Ground wheat	—43%
Wheat gluten	—10%
Alfalfa	— 3%
NaCl	— 1%

Ration I, the stock ration, was a well-balanced diet affording rapid growth and high fertility. Ration II was quite similar



BENT-NOSED AND NORMAL

Figure 8

Skulls showing the bent-nosed condition. ♀ 6 normal, ♀ 7, ♀ 9, and ♀ 11 bent. The absence of the zygomatic bone is not an anomaly as in each case it was lost while the skull was being cleaned. Bent-nose is an inherited condition which is only manifested when there are certain dietary deficiencies.

to the Steenbock and Black No. 2965 ration containing a high amount of calcium in relation to the phosphorus present, which in the absence of vitamin *D* produces severe rickets. In order to determine whether the presence of vitamin *D* would prevent bent-nose, to each 100 grams of ration II one gram of cod liver oil was added. This constituted ration III. Realizing that 3 per cent of $CaCO_3$ might make the ration so severely rachitogenic that the animals could not survive 120 days, ration IV was formulated with 1 per cent of $CaCO_3$ replaced by 1 per cent of yeast. The presence of the yeast would cause more growth chiefly by stimulating the appetite of the rats and increasing the phosphorus intake. Ration V was identical with IV except that the yeast was treated with ultra-violet light, thus adding vitamin *D* to the diet. The last two rations, VI and VII, were designed to be high in phosphorus in relation to the amount of calcium present, the reverse of the other deficient diets. Since VI was quite complex and produced no striking results, it was soon discarded and ration VII adopted.

The ingredients of the diets were finely ground and thoroughly mixed to prevent any possibility of selective feeding on the part of the rats. The rations were fed dry.

Procedure

Three strains of rats were used: the bent stock, consisting of progeny of bent × bent matings, and two control stocks. One control strain was obtained from the Wistar Institute of Anatomy, and the other consisted of black-hooded animals secured from Dr. C. A. Hoppert's nutrition laboratory at Michigan State College. It was intended at the outset that at least 50 animals of every stock would be reared on each of the seven rations, but due to a lack of animals, the Hoppert control stock was later limited to rations II and VII.

The technique employed was to rear all animals on the stock ration until they were 28 days old. At that time they were transferred to one of the deficient rations which they were fed until 120 days of age. They were then etherized and the mid-sutures of their skulls compared with a straight edge to determine whether they were normal or bent.

Rations and water were kept before the animals at all times, tap water being used since its mineral content in East

Lansing was not high. The cages were bedded with shavings throughout the experiment and kept out of any direct sunlight.

All breeding animals were fed ration I, the balanced stock ration, to insure reproduction. Some of the stock fed the deficient rations were observed to be definitely bent before they were 120 days old, and these were transferred to the stock ration to be used later for breeding.

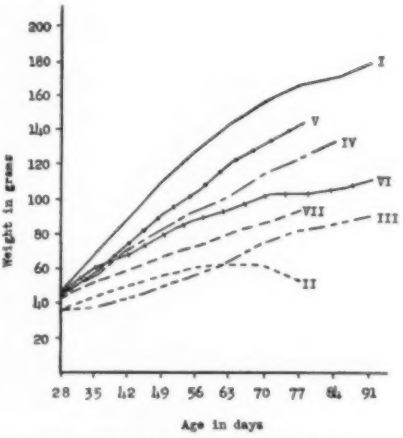
Growth

In order to determine the general response to the different diets, a number of bent animals, subsisting on the various rations, were weighed at weaning time and later at seven day intervals, and their growth curves were plotted. (Figure 9.) Several rats died after the seventh week on ration II, making it impossible to continue this curve, and consequently some of the others were also discontinued at that time.

The curves show that while ration I, the stock ration, supported the most rapid growth, ration II, the high calcium ration, was the most severe on the animals. With ration II, their average weight actually declined as much as 10.3 grams the seventh week, with a total gain of only 16.0 grams for the seven weeks, as compared with an average total gain of 118.0 grams for the animals on ration I during the same length of time. Other rations produced over the same period the following average total gains: ration III (high calcium plus cod liver oil) 45.5 grams; ration IV (yeast, high calcium) 76.0 grams; ration V (irradiated yeast, high calcium) 96.7 grams; ration VI (low calcium) 59.3 grams; and ration VII (low calcium) 49.1 grams.

Mortality

Few deaths occurred except on ration 11. The proportions dying on this ration were: 17.86 per cent of the 112 rats of the bent stock, 89.09 per cent of the 55 Wistar animals, and 78.13 per cent of the 64 Hoppert rats.



GROWTH-CURVES ON SEVEN TEST-RATIONS

Figure 9

Growth curves of rats raised from weaning on seven rations used to test the effect of diet on the incidence of bent-nose. The composition of the rations is given on page 438. Ration VII (low-calcium) produced the highest incidence of bent-nose. Ration I the least.

Relationship Between Diet and Frequency of Bent-nose

The cardinal data showing the effects of the diets on the production of bent-nose are given in Table I. The animals used here in computing the percentage of bent-nosed on each ration included only those which lived to be 120 days of age or older. There were so many deaths on ration II in both the Wistar and Hoppert stocks that the ultimate numbers were too small to give conclusive data; consequently these groups were not included in the discussion. Likewise, the twelve Wistar animals on ration VI were omitted because of the paucity of numbers.

Food was clearly a factor in determining whether or not an animal developed a bent-nose. Within the bent stock, the frequency of bent-nosed individuals ranged from practically none, on ration I, to 64.91 per cent on ration VII. The exceptional rat on ration I was an undersized individual. The bent stock of Table I consisted exclusively

of progeny from bent × bent matings, so that the distribution of genotypes affecting the nose form was probably much the same among the animals of this stock fed on each of the seven rations. It is thus clear that the difference in the incidence of bent individuals within the bent stock must have been due to the variations in diet.

The results for the Wistar stock roughly paralleled those for the bent stock and pointed to food as a factor. However, in general the incidence of bent-nose was not as high as in the corresponding group of the bent stock.

A more thorough analysis of the data revealed that the calcium-phosphorus ratio was the decisive factor in the diet. Ration II, containing 3 per cent of $CaCO_3$, raised the incidence of bent-nose from less than 1 per cent, as in ration I, to 50 per cent. The addition of vitamin *D* in cod liver oil, which tends to correct for the imbalance in the calcium-phosphorus ratio, cut the frequency of bent to 14.17 per cent, a decrease of 35.83 ± 4.88 per cent as compared with the frequency on ration II. This difference was 7.34 times its probable error. The addition of yeast to the high calcium diet had little effect on the incidence of bent. When the percentage of bent-nose among the animals on ration IV was compared with that among the animals on ration II, a difference of 4.55 ± 5.78 per cent was found, which was only 0.80 times its probable error. Such a difference might be expected, as ration IV had only 2 per cent of $CaCO_3$ while ration II contained 3 per cent. A very marked decline in the percentage of bent was again observed when vitamin *D*, in the form of irradiated yeast, was added in ration V. Irradiating the yeast alone reduced the frequency of bent from 45.45 per cent on ration IV to 3.92 per cent on ration V, a difference of 41.53 ± 4.88 per cent which was 8.51 times its probable error.

The percentage of bents obtained on ration VII showed that reversing the calcium-phosphorus ratio, making phosphorus relatively high, increased the frequency of bent-nose. However, the difference between the results from the high calcium rations and those from the low calcium ration were hardly significant in a statistical sense. Ration VII gave a percentage of 14.91 ± 5.49 per cent higher than ration II. This difference was only 2.72 times its probable error. Ration VII gave 19.45 ± 6.18 per cent more bents than ration IV, and this difference was 3.15 times its probable error. The two low calcium rations differed by 17.85 ± 7.15 per cent, a difference 2.50 times as great as its probable error. Although rations VI and VII were both low in calcium, VI was not as imbalanced as VII, since the former was a more varied diet and contained 5 per cent of milk powder which would tend to raise the calcium content.

Thus, the calcium-phosphorus ratio was a factor in producing the bent-nose. When fed a ration in which these elements were quite well balanced, practically no bent-nosed animals developed, and the frequency of bending increased, when the rats were fed a ration in which this balance had been disturbed. The introduction of vitamin *D* corrected for the imbalance, and the frequency of bending diminished. These conclusions were supported by the results from the Wistar animals.

Comparison of Growth with Incidence of Bent-nose

When a comparison was made between growth rate and the incidence of bending on the various diets among the animals of the bent stock, it was noted that in general those rations producing slight growth caused a high frequency of bending. Ration I produced the greatest gain (118 grams) in seven weeks, but less than 1 per cent of bent-noses. Conversely, animals gained only 16 grams in seven weeks on ration II, while the frequency of bending on this ration was 50 per cent. The diet producing the highest frequency of bent-nose, ration VII, caused the animals to gain on the average only 49.1 grams. Ration IV which yielded a rather high frequency of bent-nose (45.45 per cent) allowed an average increase in weight of 76

grams for the seven weeks. While vitamin *D*, when added to ration IV forming ration V, decreased the percentage of bent-nose to 3.92 per cent, it increased the average gain in weight to 96.7 grams. Ration III was exceptional. The frequency of bent-nose on this ration was 14.17 per cent, but the animals gained on an average only 47.5 grams in the seven weeks. However, it was difficult to get the animals to eat much of a ration containing cod liver oil, and this was probably responsible for the irregularity.

Bone-ash

It was desirable to make bone-ash determinations for two reasons: (1) to determine the extent of calcification of the bones induced by the deficient diets, and (2) to find out whether the various strains behaved differently toward the same ration. The bone-ash data are given in Table II.

Groups of animals from the three stocks (bent, Wistar, and Hoppert) were sacrificed after they had been fed ration II (high calcium ration) and ration VII (low calcium ration) for 20 and 40 days. The animals were etherized and the femurs removed, after which all the surplus tissue was rubbed off with cheese-cloth. Fat was extracted with hot alcohol in the conventional manner, and the bones were dried and weighed. After this they were ignited in an electric furnace to destroy the organic matter. The ash was then weighed, and the percentage of bone-ash calculated.

The percentage of inorganic matter in bone has for a long time been the principal criterion for determining the severity of rickets in rats. Femurs of normal rats vary in ash content from 45% to 60%, depending somewhat on age, sex, food and various other factors. Females tend to have a higher ash content than do males, and as animals grow older the ash content of their bones normally increases slightly. A proper calcium-phosphorus ratio in the diet, with ample vitamin *D*, tends to produce a maximum percentage of ash. However, during pregnancy and lactation the percentage of bone-ash may decrease due to the drain on the inorganic elements of the mother for the developing embryo or for the production of milk. The amount of bone-ash is markedly below the normal level in rachitic animals, often falling below 30%, depending upon the severity of the condition.

While both rations II and VII produced rickets, the high calcium ration brought about a much more extreme type than the low calcium ration. For the most part the individual percentages of bone-ash on the high calcium ration were below 30% with an extreme low of 19.58%. Feeding animals ration II beyond the 20 day period produced no significant changes in the composition of the bones.

The low calcium ration caused the individual percentages of bone-ash in most cases to drop within the rachitic range, i.e., about 40% or lower, although in no case was the percentage extremely low. The Wistar animals which had been on this low calcium ration for 40 days were on the border line between rachitic and normal animals. All three stocks showed an increase in percentage of bone-ash for the 40 day period over the 20 day period. This indicates that the physiological mechanism of these animals had begun to adjust itself to the imbalanced calcium-phosphorus ratio.

In general, there was no consistent difference in bone-ash percentage between the three strains.

Seven of the animals fed 40 days on ration II were bent-nosed when etherized. However, these animals were neither lower nor higher in bone-ash content than other members of their group, which would tend to show that the factors for bent-nose were specific rather than influencing the reaction of the whole body to the deficient diet.

Inbreeding

An inbreeding experiment was started to determine whether inbreeding with selection would increase the frequency of bending, which would be the consequence if multiple factors affected the trait. Four bent-nosed females and one

bent-nosed male (all of the same litter) were mated. They were the progeny of a bent × bent mating, and had been reared on ration VII. Nine litters, comprising 76 young, were produced.

At this stage in the experiments the data indicated that ration II — the high calcium ration — produced the highest frequency of bent-nose. So these inbred animals were fed this ration at the weaning age — 28 days. But the inbred animals did not survive the bad effects of this ration as well as animals of the bent stock had. Only 25 (32.9%) of the young lived to an age of 120 days, while of the bent stock, 82.14% survived the effects of this ration to this age. Of these 25 animals, 7, or 28%, were bent—a frequency much lower than the 50% obtained on the same ration for the non-inbred animals. It was impossible to continue the inbreeding experiment because of a lack of fertile siblings.

However, these animals were not reared in vain, for their weights proved valuable. It was desirable to know whether there was any causal connection between size at birth or gain in weight during early life and the development of bent-nose. Consequently, each animal was weighed at birth, marked, and weighed again at weaning time. Had it been found that animals which later developed bent-nose were smaller at birth than normals, one would have expected some prenatal causative factor for the trait. A low rate of gain before weaning time would have suggested some agency operating early in life.

There was no conclusive evidence of any such contributory factors. Data presented in Table III showed that there was no significant difference between the mean weights at birth of bent and normal animals. The same was true when the mean weights at weaning age and the gain in weight from birth to weaning were compared. It is worth noting, however, that the normal animals exceeded the bents in all the three weight measurements, so that in spite of the fact that the differences were not statistically significant, the matter merits further investigation with larger numbers.

Genetic Factors Causing Bent-nose

The following experiments showed that genes as well as diet were involved in the development of the bent-nose trait.

Work like Gowen's[5] may prove very enlightening in the study of inheritance of bone abnormalities, since often nutritional factors are involved. He demonstrated that separate lines of rats showed distinct differences in respect to survival on a diet deficient in vitamin *D* and high in calcium. The author has data tending to substantiate this.

Probably the most satisfactory genetic explanation of a bone abnormality in rodents is that given by Hunt, Mixter and Permar[11,14] who worked on flexed tail in the mouse. This is a condition in which the caudal vertebrae are malformed and fused, usually producing varying degrees of flexure in the tail. Although it has been definitely shown that this trait is inherited as a recessive, modifying factors are involved. The development of this character has been further studied by Kamenoff.[13]

A condition similar to flexed tail in *Mus musculus* has been reported by Huestis and Barto[10] in *Peromyscus*. It was stated that this character appeared to be due to two recessive genes.

Green and Brown,[8] in making a study of hereditary variations in the skull of the rabbit, showed that profound variations in the form of the skull could be transmitted unaltered from parent to offspring. However, no definite genetic scheme was advanced to explain the inheritance of this variation. A genetic craniometric study of two species of mice by the late C. V. Green[7] has shown that the size of the skull tends to be inherited. However, Green states that a thoroughly satisfactory genetic explanation appears impossible with the data he has gathered. Colton[3] reported work on the "high-brow" rat, but this study failed to demonstrate whether the character was inherited.

Experimental Procedure

Before the genetic experiments could be undertaken, a suitable ration had to be found. As mentioned in the preceding section, there were two requisites for this ration: (1) it could not be so severe as to cause the animals to die before they reached 120 days of age, and (2) it should produce the maximum incidence of bents among progeny of bent × bent matings. Since it was observed early that ration II (high calcium ration) produced a high incidence of bending, it was first employed in the breeding experiment, but when more data were gathered the high mortality under ration II was revealed, and it was discarded for ration VII (low calcium ration). When all the data on the different rations were analyzed, it was found that ration VII filled both of the above requisites.

A strain of rats genetically free from factors for bent-nose was needed for crossing with the bents. The Wistar strain and the Hoppert strain were tested for this purpose. The data given in Table I showed that the Wistar strain carried factors for bent-nose to a relatively high degree. On ration VII, 30.88% of these animals were bent as compared to 64.91% among those of the bent strain. The Hoppert strain proved to be nearly free from bent factors — probably as free from bent-nose as any available strain. On ration VII only 3.77% of this stock was bent. Another alternative would have been to develop a strain free from bent-nose by inbreeding and selection, but this would have extended the duration of the experiment beyond possible bounds. For these reasons, the Hoppert stock was chosen for the P_1 matings.

P_1 matings were of bent animals of the bent stock mated to normal animals of the Hoppert stock. The deficient diets rendered the animals of little use for breeding. Consequently, part of the F_1 animals were reared on ration VII to enable us to determine whether nose bending would occur in the F_1 generation, while the rest of this generation was fed the balanced diet (ration I) and mated with one another to produce the F_2 generation. Some of the latter F_1's were also back-crossed to bent-nosed individuals. Normal animals, the progeny of bent × bent, were mated to bents in an attempt to determine whether or not they carried the factors predisposing to bent-nose. Also, brother-sister matings of bents were made in order to study the change in incidence of bending afforded by inbreeding, as reported in the previous section. The care of the animals was identical with that described for the nutritional experiments.

Genetic Analysis of Data Obtained in Nutritional Studies

The data in Table I are evidence that bent-nose was influenced by genetic factors. When a group of animals was fed a deficient ration with other environmental factors constant, only a part of these developed the bent-nose condition. For instance, of the animals of the bent stock fed on ration VII, only 64.91% were bent-nosed. Thus, there must have been some innate agents causing the manifestation of bent-nose in these animals which were absent, or in some way deficient, in the remaining 35.09%. If not, one would have expected all of these animals to have been bent-nosed or normal.

However, all the groups of the bent stock on the various rations probably had approximately the same proportion of genotypes for bent-nose. Yet the different rations induced different percentages of bents. Thus, certain genetic constitutions reacted differently to different diets. An animal possessing the genes which would cause it to appear bent on the low calcium ration might have been normal if fed one of the other diets.

The fact that the three different stocks, when fed the same ration with other environmental factors nearly constant, produced different percentages of bent-nosed animals was also evidence that the character was genetic. The percentage of bent-nosed animals of the Wistar stock on ration VII was 30.88%

as compared with 64.91% for the bent stock on the same diet. The difference between these percentages was 34.03±5.66%, a difference which was 6.01 times its probable error. In the Hoppert stock on the same ration 3.77% were bent-nosed. This was 61.14±4.58% less than that of the bent stock, a difference 13.35 times its probable error, and 27.11±4.18% less than that of the Wistar stock. The latter difference was 6.49 times its probable error. Thus, it was evident that there was a difference in the innate constitution of these different stocks as to the production of bent-nose. Genes for bent-nose must have been rare in the Hoppert stock, more prevalent in the Wistar stock, and still more numerous in the bent stock.

Data Obtained in Genetic Studies

The data secured from the various crosses (P_1, F_1, back-crosses, etc.) do not reveal any simple mode of inheritance for bent-nose. Although several theories were tested, none was found that satisfactorily fits all the ratios obtained. However, the data are worth presenting. (See Table IV.)

One of the striking facts in Table IV is the decline in percentage of bent-nose with a decrease of bent-nosed ancestry. Progeny of bent × bent matings were 64.91% bent-nosed, while progeny of normal (from bent × bent) × bent matings were only 29.07% bent-nosed. The difference between these two percentages was 35.84±5.4%, a difference 6.62 times its probable error. Farther down the scale, normal (Hoppert stock) × bent matings gave progeny of which only 3.36% were bent-nosed. This percentage was 25.71±3.55% less than the percentage of bent-nosed among the progeny of normal (from bent × bent) × bent matings, and this difference was 7.24 times its probable error. With all environmental factors constant, such data afforded evidence that the character is genetic.

Unquestionably there is evidence of segregation of genes. There were only 3.36% bent-nosed among the F_1 animals from normal (of the Hoppert stock) × bent, and of the F_2's from the matings of these same F_1's, 11.73% were bent. Although this was not a high percentage of bent-nosed animals in the F_2 generation, it was significantly higher than that obtained in the F_1. The difference was 8.37±1.91%, which was 4.38 times its probable error. Likewise, there was a significant difference between the percentage of bents among the F_1's and that among the back-cross (F_1 × bent) progeny. This difference was 11.10±2.15% which was 5.16 times its probable error. The F_2 and back-cross percentages were not both consistent with any theory devised.

The data indicate that bent-nose is recessive to normal. The percentage of bents among the F_1 animals was very much lower than that expected if dominant factors were responsible for the trait. If the factors were dominant, one would expect between 50 and 100% of the F_1's to be genetically bent, and there are no good reasons for supposing that the action of ration VII would have lowered the phenotypic percentage to 3.36%. The more logical explanation is that the factors are recessive and that the Hoppert stock carried some of those factors for bent-nose. That bent-nose genes were present in the Hoppert stock was indicated by the two bent-nosed animals which appeared in the nutritional experiments.

It appears that more than one pair of genes are responsible for bent-nose. However, as with Reed's interpretation of harelip in the house mouse, (Reed[16, 17, 18]), it may be that one gene is necessary for the production of the character and the others are merely modifiers. If the character were controlled by two recessive genes, one would expect 6.25% of bents in the F_2 generation. This is not so far from the 11.73% actually obtained, yet the difference is 5.48±1.55%, which is 3.54 times its probable error. However, such a genetic scheme would not give a back-cross percentage comparable to that obtained. One would expect 25% bent-nosed in the progeny of the back-cross, and actually 14.46% was all that appeared. Three

pairs of recessive factors would give 12.5% bent-nosed among the back-cross progeny, which closely approximates the percentage obtained. Yet, among the F_2 animals, the percentage of bents was too high to justify this theory.

Another type of mating, the results of which are well worth further consideration, is that of normal animals (from bent × bent matings) × bents. Such matings were desirable in order to test whether or not such normal animals carried factors for bent-nose, and the results showed that some of these normals did carry such factors. Of the 86 progeny reared, 25, or 29.07%, were bent. This was significantly different from the 3.36% obtained when bent-nosed animals were out-crossed to the normal Hoppert stock. The difference was 25.71±3.55%, which was 7.24 times its probable error.

However, the normal animals obtained from bent × bent matings were not of the same genetic constitution as bent-nosed individuals. Progeny tests showed this. The 64.91% of bent-nosed obtained among the progeny of bent × bent matings was significantly greater than the 29.07% among the animals one of whose parents was a normal from a bent × bent matings. The difference was 35.84±5.40%, which was 6.64 times its probable error.

A part of the F_1 and F_2 animals, i.e., those born at the beginning of the genetic experiments, were put on ration II, the high calcium ration. These particular F_1 animals were kept on ration II throughout the experiment, while the F_2's were later changed to ration VII, the low calcium ration. (These data are not included in Table IV.) Of the 48 F_1 animals which were fed ration II, 19, one of which was bent, lived to 120 days of age. Also, one of the 29 which died at an earlier age was bent. The 81 F_2's which were put on ration II were transferred to ration VII early enough so that all survived to the 120 day age limit. Sixteen, or 19.75%, of these F_2's were bent. This is a higher value than that obtained among the F_2's reared from weaning on ration VII only, which is not consistent with the results of the nutritional experiment which showed that ration II produced fewer bent-nosed than ration VII.

The fact that a higher percentage of bents was obtained in these F_2's whose diet was changed from high calcium to low calcium than in those fed the low calcium ration throughout, is worth noting, although the difference between the two percentages was not quite great enough to be significant in a statistical sense. The difference was 8.02±3.36% which was 2.39 times its probable error. However, the difference is large enough to indicate that here is a possible approach to the solution of the problem. The alteration made in the feeding increased the percentage of bents in the F_2's to 19.75% which is not so far below 25%, that expected if the trait were controlled by a single pair of recessive genes. Future work on the problem could profitably be directed in search of some possible dietary changes and the proper time for making these changes which would increase the frequency of bent-nose. Thus, ratios might be obtained which would fit some genetic explanation.

Summary

1. The calcium-phosphorus balance in the food is a very important factor in determining whether bent-nose develops. That is, if the calcium-phosphorus ratio of the diet is imbalanced, bent-nose may occur. This imbalance may be attained by having either too much or too little calcium in the diet.

2. As the introduction of vitamin *D* ameliorates the deleterious effect of an imbalanced calcium-phosphorus ratio, it likewise decreases the frequency of bent-nose.

3. In general, the percentage of bent-nose produced by the various rations varied inversely with the growth rate. That is, the rations most deleterious to growth favored the development of bent-nose. On ration II, however, significant differences in weight or in gain in weight were not found between the bents and their normal siblings.

4. Bone-ash determinations failed to demonstrate any difference in bone-ash content between the three different strains which fed on a deficient ration. Ration II (high calcium ration) produced a lower percentage of bone-ash than did ration VII (low calcium ration).

5. An attempt to increase the frequency of bent-nose by inbreeding was unsuccessful, for the progeny reared on the deficient ration showed low fertility and high mortality.

6. No causal connection was found between birth weights, weights at weaning age, or increase in weight and the bent-nose trait.

TABLE I. INFLUENCE OF THE SEVEN RATIONS ON THE FREQUENCY OF BENT-NOSE.

Ration	Bent stock			Wistar stock			Hoppert stock		
	No. of animals	No. bent	% bent	No. of animals	No. bent	% bent	No. of animals	No. bent	% bent
Ration I - Stock ration	113	1	0.89	51	0	0			
Ration II - High calcium ration	92	46	50.00	6	4	66.66	14	0	0
Ration III - High calcium ration and cod liver oil	48	9	18.37	54	0	0			
Ration IV - Yeast, high calcium ration	33	85	45.45	58	42	37.93			
Ration V - Irradiated yeast, high calcium ration	53	2	3.92	53	0	0			
Ration VI - Low calcium ration	34	16	47.06	12	8	66.67			
Ration VII - Low calcium ration	98	37	41.91	68	21	30.88	51	2	3.77

TABLE II. AVERAGE PERCENTAGE OF BONE-ASH IN RATS FED RATION II AND RATION VII.

Ration	No. of days subjected to ration	Bent stock No. of animals	Bent stock Ave. % ash	Wistar stock No. of animals	Wistar stock Ave. % ash	Hoppert stock No. of animals	Hoppert stock Ave. % ash
Ration II - high calcium	20	10	28.29	14	30.60	14	30.39
Ration II - high calcium	40	9	24.90	12	25.37	11	25.36
Ration II - high calcium	60	8	23.36	5	23.86	6	32.62
Ration VII- low calcium	20	6	33.66	11	40.96	10	36.10
Ration VII - low calcium	40	7	41.00	3	47.64	8	41.62

TABLE III. COMPARISON OF MEAN WEIGHTS OF BENT-NOSED ANIMALS AND NORMAL ANIMALS OF THE INBREEDING EXPERIMENT.

	53 normal animals	25 bent-nosed animals
Mean weight at birth	6.40± .62 gms.	5.97± .63 gms.
Mean weight at weaning	39.33±6.69 gms.	35.08±6.08 gms.
Mean gain in weight	32.79±6.55 gms.	29.11±5.92 gms.

TABLE IV. FREQUENCY OF BENT-NOSE AMONG ANIMALS FROM THE VARIOUS CROSSES, ALL REARED ON RATION VII.

Type of mating	Total offspring	Number bent-nosed	Per cent bent-nosed
Bent x bent	50	37	64.91 ± 4.82
Normal (from bent x bent) x bent	86	85	98.87 ± 3.37
F_1 (bent x normal)	119	4	3.36 ± 1.11
F_2 (F_1 x F_1)	196	23	11.73 ± 1.55
Recross (F_1 x bent)	166	35	21.16 ± 1.86

7. Since ration VII (low calcium ration) produced the highest percentage of bent-nosed animals, yet did not cause death of the animals before they were 120 days old, it proved to be the most desirable ration on which to rear the animals in the genetic experiments.

8. Bent-nose in the rat is influenced by genetic factors. Different types of matings produced different frequencies of bent-nose when environmental factors were constant.

9. There is definite evidence of segregation of genes. The frequency of bent-nose in the F_2 generation is significantly greater than that in the F_1.

10. It is evident that in general the factors for bent-nose are recessive to those for normal.

11. Probably more than one pair of genes is necessary for the manifestation of bent-nose. Whether some of these factors are merely modifiers has not been determined.

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